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(71) Applicant: THE REGENTS OF THE UNIVERSITY OF MICHIGAN [US/US]; Technology Management Office, Suite 2071, Wolverine Tower, 3003 S. State Street, Ann Arbor, MI 48109-1280 (US).

(72) Inventors: WILSON, James, M.; 1350 N. Avignon Drive, Gladwyne, PA 19035 (US). ENGELHARDT, John; 805 Lawson Avenue, Havertown, PA 19083 (US).

(74) Agents: SMITH, DeAnn, F. et al.; Harness, Dickey & Pierce, P.O. Box 828, Bloomfield Hills, MI 48303 (US).

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(57) Abstract

The present invention comprises an improved adenovirus vector and methods for making and using such vectors. The adenovirus vectors of the present invention retain at least a portion of the adenoviral E3 region, carry a deletion of at least a portion of the adenoviral E1 region. Vectors of the present invention preferably also include an additional deletion to accommodate a transgene and/or other mutations which result in reduced expression or over-expression of adenoviral protein and/or reduced viral replication. The vectors of the present invention further include a transgene operatively-linked thereto. By reducing or eliminating viral replication and viral protein expression, the immune response of the infected host to the virus and viral protein is decreased and persistence of transgene expression can be increased. The adenovirus vectors of the present invention are thus particularly useful in gene transfer and therapy.

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ADENOVIRUS VECTORS FOR GENE THERAPY

SPONSORSHIP

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RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Application Serial No. 943,952, entitled "Non-Human Animal Characterized by a Human Airway," filed September 11, 1992, and also a continuation-in-part of U.S. Application Serial No. 08/067,296, entitled "Gene Therapy for Cystic Fibrosis," filed on May 25, 1993, which is a divisional of U.S. Application Serial No. 584,275, entitled "Gene Therapy Vector for Cystic Fibrosis," filed on September 18, 1990, which is a continuation-in-part of U.S. Application Serial No. 401,609, entitled "Cystic Fibrosis Gene," filed on August 31, 1989, which is a continuation-in-part of U.S. Application Serial No. 399,945, entitled "Cystic Fibrosis Gene," filed on August 24, 1989, now abandoned, which is a continuation-in-part of U.S. Application Serial No. 396,894, entitled "Cystic Fibrosis Gene," filed on August 22, 1989, now abandoned, all of which applications are specifically incorporated by reference herein.

FIELD OF THE INVENTION

The present invention relates generally to adenovirus vectors and to methods for making and using such vectors, particularly to an adenovirus vector containing at least a portion of the E3 region and a deletion of at least a portion of the E1 region, and more particularly to such an adenovirus vector containing an additional deletion or mutation.

BACKGROUND OF THE INVENTION

Adenoviruses are a large family of double-stranded DNA viruses that replicate in the nucleus of the host cell. The viral genes are categorized as either "early" or "late" genes. These temporal categories are based on when the genes are transcribed into mRNA during the virus life cycle. Transcription occurs coordinately and the transition from early to late transcription occurs at approximately 10 hours post-infection, coinciding with DNA replication. As the viral genes are expressed, there is a gradual reduction in host cell RNA, DNA and protein synthesis while the quantity of viral proteins and nucleic acids slowly rises. By about 36 hours post-infection, the host cell disintegrates and the virus is released into the environment.

Adenoviruses are thus ideal candidates for generating vectors useful in gene therapy because the virus uses the host cell's own machinery to synthesize viral RNA, DNA and proteins. Furthermore, the transcription of the adenovirus genes, the organization of the genome and the DNA sequence of the genome have been well defined. Thus, non-viral DNA encoding proteins of interest can be inserted into the adenovirus genome at appropriate locations, and these proteins can be readily expressed in the host cell.

Because adenovirus replication ultimately results in cell death, previous adenovirus vectors were designed to reduce virus replication. Reduced viral replication has been accomplished by deleting or mutating portions of early genes such as the E1a/E1b region, as this region of the genome regulates the expression of various other adenovirus genes required for DNA replication. Berkner, K.L., *Biotechniques* 6:616-629 (1988). Horwitz, M.S., "Virology," 2d ed., Raven Press Ltd., p.1679-1720 (1990).

Although E1a/E1b-deleted adenoviruses exhibit reduced virus replication, these vectors are inefficiently packaged into the viral capsid due to the large genome generated by the additional transgene DNA. The inefficient packaging reduces the titer of the virus stocks by 2-3 logs in comparison to traditional vectors. The low titer of the E1a/E1b-deleted viruses reduces their usefulness, especially for applications to entire organs such as the lung.

Vectors have been constructed with an additional deletion in the E3 region. This deletion increases the amount of non-viral DNA that can be inserted into the vector while maintaining efficient packaging of the recombinant virus. Engelhardt, J.F. et al., *Nature Genet.* 4:27-34 (1993). However, there is speculation that expression of the E3 gene aids virus-infected cells in avoiding the immune response of the host. Therefore, deleting the E3 region is undesirable, as the lack of E3 protein expression increases the chance that the virus infected cells will be rejected by the immune system of the host.

E3-inclusive, E1a/E1b-deleted adenovirus vectors currently exist and have been approved for clinical trial. A major disadvantage of these vectors, however, is again, inefficient packaging because of large genome size that leads to much lower titers than traditional vectors, making them less useful in large scale human applications. With deletions in other regions of the adenovirus genome, the E3 region could be retained and the appropriate viral genome size could be achieved for the production of high titer stocks for clinical use.

A particularly useful application for adenovirus vectors is in the treatment of cystic fibrosis (CF) by gene therapy. Various gene therapy approaches have been considered for cystic fibrosis without sufficient results. One such approach is to selectively reconstitute cystic fibrosis transmembrane regulator (CFTR) gene expression in the surface epithelium using gene transfer substrates delivered directly into the airway. Although transfection of airway epithelial cells has been achieved *in vivo* with cationic liposomes, efficiencies have been below what is required for therapeutic efficacy. Hazinski, T.A. et al., *Am. J. Respir. Cell. Mol. Biol.* 4:206-209 (1991); Yoshimura, K. et al., *Nucleic Acids Res.* 20:3233-3240 (1992). Likewise, recombinant retroviruses carrying the CFTR gene have been unacceptable because efficient and stable recombinant gene expression can be accomplished in proximal airway with recombinant retroviruses only if the epithelium is undifferentiated and regenerating at the time of exposure to virus, a situation that is difficult to simulate in patients. Engelhardt, J.E. et al., *J. Clin. Invest.* 90:2598-2607 (1992).

The use of recombinant adenoviruses for cystic fibrosis gene therapy is thus particularly attractive especially considering the important advantages of adenovirus vectors, including their natural tropicity to human airway, growth to extremely high titers, and their ability to transfer and express recombinant genes in nondividing cells. Graham, F.L. et al., "Gene Transfer and Expression Protocol," E.J. Murray ed., The Human Press, Inc., Clifton, N.J. 109-128 (1992). Due to these advantages, recombinant adenoviruses have been used to transfer genes for α-1-antitrypsin and CFTR into lungs of cotton rats. Rosenfeld, M.A. et al., *Science* 252:431-434 (1991); Rosenfeld, M.A. et al., *Cell* 68:143-155 (1992).

It would thus be desirable to produce an improved recombinant adenovirus vector. It would also be desirable to produce a recombinant adenovirus vector which has a deletion of at least a portion of the E1 region, retains at least a portion of the E3 region and contains an additional deletion to accommodate a transgene and/or other mutations which result in reduced expression or over-expression of adenoviral protein and/or reduced viral replication. It would further be desirable to produce a recombinant adenovirus vector which has a deletion of at least a portion of the E1 region, retains at least a portion of the E3 region, contains an additional deletion to accommodate a transgene and/or other mutations which result in reduced expression or over-expression of adenoviral protein and/or reduced viral replication, and which contains a transgene of interest, for example, the CFTR gene. In addition, it would be desirable to produce a gene expression system useful for the

study and employment of therapeutic approaches.

SUMMARY OF THE INVENTION

The present invention comprises an improved adenovirus vector and methods for making and using such vectors. The vectors are particularly useful in gene transfer and expression. The vectors of the present invention are also useful as a research model to study gene expression and various therapeutic approaches. The adenovirus vectors of the present invention reduce or eliminate viral replication *in vivo* and viral protein expression, thereby decreasing the immune response of the infected host to the virus and viral protein. By decreasing the host immune response, the persistence of expression of the inserted gene is increased. The adenovirus vectors of the present invention are efficiently packaged to further facilitate the transfer of inserted non-viral genes to host cells. Thus, the vectors of the present invention may be characterized by efficient packaging, reduced virus replication and increased persistence of transgene expression.

In one embodiment of the present invention adenovirus vectors retain at least a portion of the E3 region and carry a deletion of at least a portion of the E1 region, which is upstream from the E3 region, as well as a deletion within adenovirus genes other than E1 and E3 region genes to reduce viral genome size. By up and downstream is meant the location on a nucleotide sequence in the conventional 5′ to 3′ orientation, wherein upstream is towards the 5′ end and downstream is towards the 3′ end of the sequence. Deletions in the E3 gene region are presently based on available restriction sites for cloning; however, it will be appreciated that other E3 deletions are also contemplated by the present invention. Deletions in genes other than in the E1 and E3 genes may occur in structural or nonstructural viral genes and can affect the early or late genes, and include, in particular, deletions in the E2a region. These deletions allow for the retention of at least a portion of the E3 region and inclusion of a transgene. The inclusion of a portion of the E3 region increases persistence of transgene expression.

In another embodiment of the present invention, the adenovirus vectors contain at least a portion of the E3 region and a deletion of at least a portion of the E1 region, as well as a mutation which produces temperature-sensitive (ts) virus. The mutation imparting temperature-sensitivity to these vectors can occur in adenovirus genes encoding nonstructural proteins or structural proteins, or both. Viral stocks of the vectors are capable of replicating *in vitro* at permissive

temperatures but are incapable or have reduced ability to replicate *in vivo* at non-permissive temperature, thus increasing transgene expression.

In an additional embodiment of the present invention, the temperature-sensitive adenovirus vectors are replication-defective virus at both permissive and non-permissive temperatures. These vectors contain a mutation in non-structural genes or structural genes, or both, such that the mutant viral proteins are unstable at both permissive and non-permissive temperatures.

In another embodiment of the present invention, the recombinant adenoviruses contain at least a portion of the E3 region and a deletion of at least a portion of the E1 region, as well as mutations in other genes producing viral proteins. These vectors destabilize expression of viral proteins that are expressed or over-expressed in E3-inclusive/E1-deleted vectors, thereby decreasing the immune response of the infected host to the viral protein. Vectors of the present invention which contain at least a portion of the E3 region, carry a deletion of at least a portion of the E1 region and include a deletion within adenovirus genes other than E1 and E3 genes, and/or a mutation which results in increased persistence of transgene expression, reduction in expressed or over-expressed adenoviral protein and/or reduced viral replication, are hereinafter referred to as "second generation" vectors. Second generation vectors are preferably temperature-sensitive to allow for *in vitro* propagation of virus at 32°C and defective growth *in vivo* at 37°C. The vectors of the present invention which contain at least a portion of the E3 region and a deletion of at least a portion of the E1 region are herein referred to as "first generation" vectors.

In a further embodiment of this invention the adenovirus vectors carry a transgene operatively-linked thereto. By "operatively-linked" is meant attached in a manner which allows for transgene transcription, *e.g.*, through the use of sufficient regulatory elements in the vector construct. It will be appreciated that a variety of strategies and the methodology for creating such constructs are well known to those skilled in the art. By "transgene" is meant any gene or gene region that is foreign to the naturally occurring adenovirus. By "gene" is meant any nucleic acid or reverse transcript thereof having a sequence which codes for the polypeptide or protein of interest, including those which function as structural or regulatory elements, or both. This term includes nucleic acids having naturally-occurring sequences, as well as synthetic or any coding sequences which are capable of expression. Although any number of transgenes can be employed in the practice

of the present invention, preferred transgenes include those useful for gene therapy, such as *e.g.* the gene for cystic fibrosis transmembrane regulator (CFTR). Thus, in an additional embodiment of this invention the adenovirus vectors are used to treat non-inherited and inherited genetic and epigenetic diseases or disorders.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1(A-B) are bar graphs showing the distribution of cell types in several proximal airway samples.

Figures 2(A-E) are graphs showing the recovery of recombinant virus in xenograft effluents.

Figure 3 is a map of two potential temperature-sensitive mutations in adenoviral replication.

Figure 4 is a photomicrograph of an Ad.CMV*lacZ* infected baboon lung stained *in situ* for β-galactosidase with Xgal.

Figures 5(A-B) are photomicrographs of Xgal stained tissue from CFTR and *lacZ* infused lobes of the CFB4 animal.

Figures 6(A-B) are photomicrographs of Xgal stained tissue from CFTR and *lacZ* infused lobes of the CFB4 animal.

Figures 7(A-B) are photomicrographs showing transgene localization in bronchial brushings of the CFB4 animal.

Figures 8(A-C) are graphs showing the effects of gene transfer on hematologic values.

Figure 9 is a graph showing the effects of gene transfer on the difference between alveolar and arterial oxygen tensions.

Figure 10 is a schematic showing the location and extent of chest radiographic abnormalities detected before and after gene administration.

Figure 11 is a graph showing bronchoalveolar lavage fluid differential cell counts before and after gene administration.

Figure 12 is a schematic showing the location and extent of lymphocytic pneumonitis in the lungs of animals following gene administration.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Construction of the adenovirus vectors of the present invention is performed by standard tranfection techniques for E1a/E1b-deleted adenovirus vectors using the complementation human embryonic kidney (HEK) 293 cell line, a human kidney cell line containing a functional E1a gene which provides a transacting E1a protein, allowing efficient replication of the adenovirus vectors. Three general techniques are

used in generating recombinant stocks: 1) overlapping fragments of the viral genome, one containing the mutant gene and a second that contains the remainder of the wild type genome, are cotransfected using standard $CaPO_4$ transfection techniques in 293 cells followed by viral plaqueing with agar overlay; 2) ligation of various regions of the viral genome containing mutated regions and nonmutated regions followed by standard $CaPO_4$ transfection techniques; and 3) cotransfection of mutant strain viral genomic DNA and plasmid sequences containing the transgene and partial viral sequences for homologous recombination.

Mutant viral stocks which contain no transgene are obtained from previously published work or generated and selected using standard recombinant molecular cloning techniques known and practiced by those skilled in the art. Mutant viral stocks obtained from previously published work are combined with other deletions necessary for the generation of recombinant virus by the above-stated techniques. Deletions, insertions, and other mutations are generated using standard techniques and selection schemes. As one of skill in the art will recognize, "mutation" refers to any alteration of the DNA including, but not limited to, deletions, insertions, and missense and nonsense mutations. One skilled in the art will also appreciate which type of mutation is being referred to from the context in which it is used.

The human bronchial xenograft model of human airway as described in the parent U.S. Application Serial No. 943,952, entitled "Non-Human Animal Characterized by a Human Airway", filed September 11, 1992, has also been used to study the adenovirus vectors of the present invention. The xenografts develop into a fully differentiated pseudostratified epithelium that is indistinguishable from that found in the endogenous human airway. This model therefore provides a unique opportunity in which to assess the ability of adenovirus vectors to replicate in human airway epithelium. To test for increased persistence of transgenes, two additional animal models are used, the ferret and non-human primates.

As further discussed below, immunocytochemical detection of three adenovirus proteins (hexon, fiber and the DBP E2a gene product) within the human bronchial xenograft model after infection with recombinant adenoviruses showed that the two late viral proteins hexon and fiber were not expressed at detectable levels, while the 72kd DPB E2a gene product was expressed at higher than wild type level (*i.e.* higher than xenografts infected with wild type adenovirus type 5). These data are the foundation for targeting specific genes (*i.e.* the 72kd DPB) for mutation or deletion which are known to be over-expressed *in vivo.*

The adenovirus vectors of the present invention are useful for gene therapy to treat various non-inherited or inherited genetic or epigenetic diseases or disorders such as adult respiratory distress syndrome (ARDS), cystic fibrosis and asthma. The adenovirus vectors of the present invention are also useful as a research model to study therapeutic approaches including gene therapy. As one of skill in the art will realize, each adenovirus vector of the present invention may be constructed having any transgene useful in the treatment of various diseases operatively-linked thereto.

When used as a therapeutic, a therapeutically effective dosage of the vectors of the present invention will be administered for a therapeutically effective duration. By "therapeutically effective amount" and "therapeutically effective duration" is meant an amount and duration sufficient to achieve a selected desired result in accordance with the present invention without undue adverse or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. A therapeutically effective human dosage of the vectors of the present invention is believed to be in the range of from about 20 to about 50 ml of saline solution containing concentrations of from about 1 x 10^7 to 1 x 10^{10} pfu/ml vector of the present invention. A preferred human dosage is about 20 ml saline solution at the above concentrations.

It will be appreciated that formulations suitable for administration of the adenovirus vectors of the present invention include aqueous and non-aqueous isotonic sterile injection solutions and aqueous and non-aqueous sterile suspensions. In the case of CFTR gene delivery, preferred solutions for bronchial instillation are sterile saline solutions containing in the range of from about 1x10^7 to 1x10^{10} pfu/ml, more particularly, in the range of from about 1x10^8 to 1x10^9 pfu/ml of the viral vector of the present invention. It will also be appreciated that administration of the adenovirus vectors of the present invention will be by procedures well established in the pharmaceutical arts, *e.g.* by direct delivery to the target organ, tissue or site, intranasally, intravenously, intramuscularly, subcutaneously, intradermally and through oral administration, either alone or in combination.

SPECIFIC EXAMPLE 1 - Transgene Expression

In accordance with the principles of the present invention, exposure of the xenograft to recombinant adenoviruses results in transgene expression (*i.e. lacZ* and human CFTR) in a large number of surface epithelial cells (*i.e.* 5-20% with concentrated virus) and the expression is stable and not associated with pathology. Recombinant gene expression was detected in all cell types of the surface

epithelium except basal cells. A possible explanation for the low level of expression in basal cells may simply relate to the level of abundance of adenoviral receptors on this cell type.

Immunocytochemical techniques were used to detect adenoviral proteins in cells of the xenograft that express the recombinant gene. Horwitz, M.S., "Adenoviridae and their Replication In: Virology," B.N. Fields, D.M. Knipe et al., eds., 2d ed., Raven Press, Ltd., N.Y. 1679-1721 (1990). Expression of several adenoviral genes was evaluated including 1) the E2a gene, expressed in the early and late phase of the adenoviral life cycle, which encodes a 72 kd DNA binding protein; and 2) the L3 and L5 transcripts, which are formed from the single late transcriptional unit, and encode the structural proteins hexon and fiber, respectively. The program of adenoviral protein expression in cells of the human xenograft differed substantially between wild type Ad5 and the E1-deleted recombinants. Cells infected with wild type Ad5 expressed high levels of the structural proteins hexon and fiber and lower levels of the E2a gene product indicating they are capable of supporting the full life cycle of Ad5. Cells harboring E1-deleted Ad5 expressed little if any of the structural proteins hexon and fiber; however, the E2a gene was expressed at very high levels in a subset of transgene containing cells. This suggests that the recombinant virus is prevented from transitioning into the late phase of transcription in the absence of E1a and E1b. However, a subset of cells is capable of activating transcription from the E2a promoter independent of E1a and E1b. The consequences of E2a expression in human airway epithelial cells are presently unknown. Non-human primates in which both the *lac*Z and CFTR adenoviruses were administered into the airway express high levels of DBP in a subset of *lac*Z expressing cells, but do not generate an immune response to this protein product of the E2a gene.

Xenografts were sequentially irrigated for a 3-week period after exposure to adenovirus, and the effluents were analyzed for wild type and recombinant virus. Wild type virus was never detected in the effluents and the concentration of recombinant virus dropped precipitously during the initial week and stabilized at low but detectable concentrations for the second phase of the experiment, which lasted up to 24 days. It is possible that the virus recovered in the effluents represents residual virus from the initial infusion. An alternative explanation is that the genetically reconstituted xenografts support low levels of virus production. Replication of E1a-deleted viruses has been described *in vitro*. Horwitz, M.S., *supra*. One potential mechanism to account for the presence of virus in effluents is that an

occasional cell in the xenograft overcomes the block in adenoviral replication, leading to its death and the production of more recombinant virus. The life cycle of group C viruses such as Ad2 and Ad5 in the context of full E1 expression is extremely efficient and results in the production of 10,000 virions per infected cell. Green, M. et al., *Virol.* 13:169-176 (1961). Progression of the full Ad lytic cycle in only 1-2 cells per xenograft per week could account for the steady state level of recombinant virus detected in the effluents (*i.e.* 100 to 10,000 viruses/irrigation). Another potential mechanism is that the virus replicates at low levels in a large population of infected cells and low quantities of virus are released when the cells undergo normal turnover. The inability to detect wild type levels of structural proteins in a sample of 1500 infected cells is consistent with either mechanism.

An important outcome of this example as it relates to the utility of recombinant adenoviruses for gene therapy is the stability of recombinant gene expression that was achieved in the human xenograft. Detailed characterization of the molecular state of the viral genome in xenografts is difficult because of the limited amount of material available for analysis. DNA analysis of cultured human epithelial cells infected with the *lac*Z and CFTR viruses indicated that the adenoviral genome persists primarily as nonintegrated DNA (data not shown). Additionally, episomal persistence of recombinant Ad.RSVβgal has been shown in newborn mice. Stratford - Perricaudet, L.D. et al., *J. Clin. Invest.* 90:626-630 (1992). This finding suggests that the persistence achieved in the xenograft may be due to extrachromosomal viral genomes that are stabilized or replicating in the absence of virion formation. An alternative explanation is that the apparent persistence of recombinant gene expression is due to ongoing production of virus and reinfection. This possibility is unlikely, however, because the levels of virus recovered in the effluents is 5 to 6 logs lower than that necessary to sustain the observed level of stable genetic reconstitution.

Preparation of recombinant adenovirus. Four different replication-defective adenoviruses based on Ad5 were used, including Ad.E1Δ, Ad.CMV*lacZ*, Ad.CB-CFTR, and Ad.RSVβgal. Ad.E1Δ had been deleted of E1a sequences spanning 1.0 to 9.2 map units (mu) and E3 sequences spanning 78.4 to 86 mu. A minigene containing the cytomegalovirus (CMV) promoter, cytoplasmic *lacZ* gene, and SV40 poly A was introduced at the site of the E1 deletion of Ad.E1Δ to make Ad.CMV*lacZ*. The structure of Ad.RSVβgal has been described previously. Stratford-Perricaudet, L.D. et al., *J. Clin. Invest.* 90:626-630 (1992). In this virus, E1 sequences from 1.3

to 9.4 mu have been deleted and replaced with a minigene containing the Rous sarcoma virus long terminal repeat (RSV LTR), *lacZ* gene with nuclear localization sequences, and SV40 early region polyadenylation signal; in addition, E3 sequences spanning to 78.5 to 84.7 mu have been deleted. Ad.CB-CFTR is a derivative of Ad.E1Δ in which the following minigene has been inserted into the E1a deletion site: CMV enhancer, β-actin promoter, human CFTR cDNA and SV40 poly A.

Stock of recombinant viruses were prepared as follows. Cultures of 293 cells (30 x 150 mm plates) grown in DMEM containing 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin were infected at 80% confluency at a MOI of 5 pfu per cell. Cells were harvested by centrifugation 30 hr at post-infection. The pellets were resuspended in a final volume of 18 ml of 10 mM Tris pH 8.0 and subjected to three rounds of freeze-thaw, followed by separation of cell debris by centrifugation at 1500 x g for 20 min. Crude viral supernatants were layered onto a CsCl step gradient and centrifuged for 2 hr at 50,000 x g. The intact viral particles were subjected to a second round of CsCl banding such that the final CsCl purified adenovirus contained 3-6 x 10^{13} viral particles (as measured by OD at 260 nm) in 500-700 μl. Concentrated viral stocks were desalted by gel filtration through Sephadex G50 in Ham's F12 medium to yield a final purified stock of 1-2 x 10^{13} viral particles/ml. Viral titers as measured by plaque formation on 293 cells yielded stocks ranging from 0.2-2 x 10^{12} pfu/ml. Viral stocks were used for infusion into xenografts immediately after completion of the purification. All stocks were evaluated for the presence of replication competent adenovirus by infection at a MOI of 100 onto HeLa cells and passaging the cells for 30 days. Presence of replication-competent virus in the original stock would manifest itself as the development of cytopathic effects (CPE) in the HeLa cells. None of the stocks used in these experiments yielded such effects.

Generation of human bronchial xenografts. Primary human bronchial epithelial cells were harvested from the mainstem bronchi of lungs (at least three samples for each vector analyzed) destined for transplantation using modifications of a previously described protocol. Yankaskas, J.R. et al., *Lancet* 1:954-956 (1985). Dissected airways were rinsed with MEM containing 50 U/ml penicillin, 50 μg/ml streptomycin, 40 μg/ml tobramycin, 50 μg/ml ceftazidine, 2.5 μg/ml amphotericin B, 10 μg/ml DNAse, and 0.5 mg/ml DTT for 4-12 hr at 4°C. Tissue was then placed in the same media supplemented with 0.1% protease-14 and incubated for an additional 30-34 hr at 4°C. Following the addition of FCS to a final concentration of

10%, the cells were harvested by agitation and blunt scraping. Cells were pelleted and washed twice in Ham's F12 containing 10% FCS and plated at 2 x 10^6 cells/100 mm dish in Ham's F12 containing 1 μM hydrocortisone, 10 μg/ml insulin, 30 Nm thyroxine, 5 μg/ml transferrin, 25 ng/ml epidermal growth factor, 3.75 μg/ml endothelial cell growth supplement, 10 ng/ml cholera toxin, 50 U/ml penicillin, 50 μg/ml streptomycin, 40 μg/ml tobramycin, 50 μg/ml ceftazidine, and 2.5 μg/ml amphotericin B. The medium was replaced after 36 hr and changed every 24 hr thereafter. On the fourth day, cells were harvested by treatment with 0.1% trypsin followed by the addition of 10% FCS/Ham's F12 and resuspended at a concentration of 1 x 10^6 cells per 25 μl in hormonally defined medium in preparation for seeding.

Open-ended grafts were generated from rat tracheas removed from 200 to 250 gm male Fisher 344 rats and subjected to three rounds of freeze-thaw as previously described. Engelhardt, J.F. et al., *Proc. Natl. Acad. Sci.(USA)* 88:11192-11196 (1991). Cells (1 x 10^6) were injected into the lumen of denuded rat tracheas followed by ligation of the tracheal ends to flexible plastic tubing. These seeded xenografts were transplanted subcutaneously into the flanks of *nu/nu* mice such that the ends of the tubing exited through the back of the neck. Grafts were allowed to regenerate for three to four weeks before infusion of adenovirus. Stocks of adenoviruses in Ham's F12 (1ml) were infused into the xenografts over the course of 1 hr and excess fluid was subsequently removed from the lumen by expulsion with air.

Electron microscopic and morphometric analysis of xenografts. Xenografts were excised and fixed as described. Engelhardt, J.F. et al., *Proc. Natl. Acad. Sci. (USA)* 88:11192-11196 (1991). Following fixation, the tissue was washed repeatedly in 0.1M cacodylate, postfixed in 1% osmium tetroxide, dehydrated in alcohol, and embedded in epoxy resins. Sections were stained with uranyl acetate and lead citrate before being viewed and photographed in a Philips CM10 electron microscope.

Morphometric analysis of cell types contained within a donor bronchus and three xenografts generated from this tissue were performed to assess the extent of epithelial reconstitution within this xenograft model. Cells were categorized based on morphologic criteria into four groups: ciliated cells by possessing apically localized cilia, goblet cells by the presence of electron lucent secretory granules, intermediate cells by no luminal contact with cytoplasm extending at least one third the height of the epithelium and not fulfilling the criteria of basal cells, and basal cells

by the presence of tonofilaments and a high nuclear to cytoplasmic ratio with the majority of cytoplasm residing on the basal lamina. At lease 30 independent fields from 5 blocks were analyzed from donor bronchus to give a total of 1500 cells. Each of three independently generated xenografts was embedded into four blocks and one complete cross-section of each of these blocks was analyzed giving a total of 12 independent regions of the xenografts. In total, 3000 cells were analyzed from three xenografts.

Cytochemical and immunocytochemical analysis of xenografts for β-galactosidase, CFTR, cytokeratins, and adenoviral proteins. Cytochemical localization and characterization of grafts for β-galactosidase activity by light microscopy was performed with glutaraldehyde-fixed tissue stained in Xgal for 4 hr followed by embedding in GMA as described previously. Engelhardt, J.F. et al., *Proc. Natl. Acad. Sci. (USA)* 88:11192-11196 (1991). The abundance of *lacZ* transgene-expressing cells was quantitated by counting the percentage of Xgal positive cells from GMA sections within a group of 16,000 cells. The distribution of the various cell types (ciliated, basal, goblet, and intermediate cells) within the xenograft epithelium was based on averages from 3000 cells counted from 5 independent regions of a representative criteria. Identification of the various cell types was based on the following morphologic criteria: ciliated cell - the presence of cilia; basal cell - cuboidal appearing cell of high nuclear to cytoplasmic ration with nuclei in the lowest layer of epithelium, direct contact with the basal lamina, and no luminal contact; goblet cells - the presence of mucous granules as visualized under Nomarski optics; and intermediate cells - cells in contact with the basal lamina but with cytoplasm extending upward into the epithelium but not contacting the luminal surface. The relative infectivity of ciliated cells, basal cells, goblet cells, and intermediate cells was quantitated by counting 1000 Xgal positive cells from GMA sections of grafts infected with $1x10^{12}$ pfu/ml of virus. Distribution of cells expressing *lacZ* was also evaluated by immunocytochemical co-localization with a cell specific marker to basal cells, (cytokeratin 14), and one to differentiated columnar cells (cytokeratin 18). Randell, S.H. et al., *Am. J. Respir. Cell. Mol. Biol.* 4:544-554 (1991). Rutten, A.A.J.J.L. et al., *Virchows Archiv. B. Cell. Pathol.* 56:111-117 (1988).

Immunocytochemical co-localization of β-galactosidase, cytokeratin 14, and cytokeratin 18 proteins was performed as follows. Sections of fresh frozen tissue (6 μm) were postfixed in methanol for 10 min, air dried, and blocked in PBS containing 20% donkey serum (DS) for 30 min. Sections were then incubated sequentially in

undiluted hybridoma supernatant to cytokeratin 14 (gift from Dr. Ramaekers, RCK107) for 90 min followed by three 8 min washes in 1.5% DS/PBS and incubation in 5 μg/ml of AMCA-antimouse $(Fab')_2$ secondary antibody for 30 min. After washing, these sections were incubated in PBS/1.5% DS containing 66 μg/ml rabbit anti-β-galactosidase (5'-3' inc.) and FITC-cytokeratin 18 (Sigma) at a dilution of 1:400 for 90 min. Sections were washed and incubated in 5 μg/ml donkey anti-rabbit Texas Red for 30 min. Following three washes in 1.5% DS/PBS, sections were mounted in Citifluor antifadent and visualized on a Microphet-FXA Nikon fluorescent microscope. Cell types expressing *lacZ* were quantitated from sections stained for β-galactosidase, cytokeratin 14 and cytokeratin 18; 1000 total *lacZ* positive cells were counted. Localization of CFTR was performed using an antibody to the 13 C-terminal amino acids of human CFTR (α1468) as previously described. Engelhardt, J.F. et al., *Nature Genet.* 2:240-248 (1992).

Immunocytochemical co-localization of β-galactosidase with the adenoviral proteins DBP, fiber, and hexon was performed as with cytokeratin co-localization using the following modifications. Sections were incubated sequentially with 66 μg/ml anti-β-galactosidase (5'→3', Inc), a 1/10 dilution of hybridoma supernatant to Ad5 DBP, 5 μg/ml of both donkey anti-rabbit-AMCA and donkey anti-mouse Texas Red, followed by a 1/10 dilution of mouse anti-Ad3 fiber-FITC (Ab805F, Chemicon, Inc.). Western analysis of purified adenovirus type 5 indicated that Ab805F recognizes a 62 kd protein consistent with fiber protein. Additional sections were treated similarly by replacing the Ab805F with a goat anti-Ad5-FITC antibody to the hexon protein of adenovirus type 5 (Ab1059F, Chemicon, Inc.).

In situ detection of CFTR mRNA. Frozen sections (6 μm) were mounted on gelatin poly(L-lysine)-coated slides and fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for *in situ* hybridization analysis as previously described using ^{35}S RNA probes to the R-domain (1899bp to 2623bp) of human CFTR. Engelhardt, J.F. et al., *Nature Genet.* 2:240-248 (1992). As a control, samples were also assessed for hybridization to an antisense CFTR probe. In addition, samples were hybridized to the sense or antisense CFTR probe both with and without pretreatment with RNAse.

Recovery of adenoviruses from xenografts. To assess the ability of recombinant adenovirus to replicate within human xenograft epithelium, effluent fractions were collected at timed intervals following infection. Xenografts were infected with freshly prepared stocks of virus ($1x10^{11}$, 10^{10}, and 10^9 pfu/ml) for 16 hr

followed by washing with two 1 ml aliquots of buffered saline. The second aliquot was designated fraction 1. At 3 1/2-day intervals additional fraction were collected by irrigating the lumen of the xenograft with 1 ml aliquots of buffered saline. All fractions were frozen on dry ice and stored at -80°C. Upon completion of the experiment the fractions were thawed and evaluated for recombinant virus by a limiting dilution plaque assay on 293 cells. Plaques were stained for β-galactosidase by overlaying 1 ml of Xgal solution onto the agar at day 9 following infection. All plaques showed the presence of blue Xgal precipitate.

Characterization of the surface epithelium reconstituted in xenografts. Xenografts seeded with 1×10^6 freshly isolated human bronchial epithelial cells gave rise to fully differentiated epithelia within 3 weeks after implantation into *nu/nu* mice. Transmission electron microscopy of epithelium from a xenograft seeded with human bronchial epithelial cells harvested at 42 days, demonstrated that the general organization of epithelia in xenografts is similar to that found in native airway. Electron micrographs were analyzed morphometrically to evaluate the distribution of cell types found in proximal surface epithelia of these tissues. A summary of these data are included in Figure 1A. The distribution of cell types found in xenografts and bronchial tissues closely resembles that previously described for the proximal airway of primates which is also shown in Figure 1A. Plopper, C.G. et al., *Am. J. Anat.* 184:31-40 (1989); Wilson, D.W. et al., *Am. J. Anat.* 171:25-40 (1984). Furthermore, there were no differences in the abundance of ciliated cells, goblet cells, basal cells and intermediate cells noted between the xenografts and bronchial tissue from which the xenografts were derived (see Figure 1A for statistical analysis). This finding confirms the validity of the xenograft model for studying proximal human airway.

Adenoviral-mediated gene transfer in human bronchial epithelia. A variety of recombinant adenoviruses based on Ad5 were used. E1 and E3 sequences were deleted from each virus. Some of the recombinants contained a minigene in place of E1. Viruses used include: Ad.E1Δ, the precursor recombinant virus in which E1 and E3 have been deleted, without addition of any other sequences; Ad.RSVβgal, containing nuclear targeted *lacZ* expressed from a Rous Sarcoma virus (RSV) LTR; Ad.CMV*lacZ*, containing cytoplasmic *lacZ* expressed from the cytomegalovirus (CMV) promoter; and Ad.CB-CFTR, containing human CFTR expressed from the CMV enhancer and β-actin promoter.

Xenografts were infected with purified stocks of Ad.RSVβgal ($1x10^{12}$, 10^9, and 10^8 pfu/ml) or Ad.E1Δ ($1x10^{12}$ pfu/ml) for one hour after which the virus was expelled. Grafts were harvested, fixed, stained in Xgal and visualized *en face* through a dissecting microscope in order to assess the overall efficiency of infection. All xenografts were harvested at 3 days post-infection except for that infected with $1x10^9$ pfu/ml, which was harvested 21 days after infusion of virus. Xenografts infected with Ad.E1Δ demonstrated no Xgal positive cells, while large areas of *lacZ* expression were demonstrated in grafts exposed to Ad.RSVβgal ($1x10^{12}$ pfu/ml) and harvested 3 days later. Morphometric analysis of GMA sections of this xenograft indicated gene expression in 11 +/-6.3% of the epithelial cells. Similarly high levels of infection were obtained with viral stocks diluted 10 to 100-fold; infection of 12 xenografts generated from 4 independent tissue samples with $1x10^{10}$ to 10^{11} pfu/ml of Ad.RSVβgal resulted in *lacZ* expression in 5-20% of the cells. The inability to achieve an increment in gene transfer at titers of virus greater than $1x10^{10}$ pfu/ml suggests that saturation of the adenoviral receptor has been achieved. Xenografts infected with Ad.RSVβgal at $1x10^9$ and 10^8 pfu/ml and examined 3 days later demonstrated gene expression in 1.9 +/-0.2% and <0.1% of the total cells of the epithelium, respectively. To determine if *lacZ* expression is stable within the bronchial epithelium, xenografts were also harvested 21 days following infection with $1x10^9$ pfu/ml Ad.RSVβgal adenovirus. No changes in the percentage of Xgal positive cells were seen between 3 days and 21 days post-infection. Transgene expression is not diminished in xenografts harvested up to 5 weeks after infection.

A series of analyses were performed to determine the distribution of transgene expression in the xenografts. Xgal-stained sections of xenografts infected with $1x10^{12}$ pfu/ml of Ad.RSVβgal were analyzed by light microscopy to determine the percentage of each cell type that expressed the transgene. These results are summarized in Figure 1B. The distribution of all cell types in the xenograft determined by light microscopy was identical to that established using ultrastructural criteria. The proportion of cells containing Xgal precipitate paralleled the distribution of cell types in the graft with the exception that very few basal cells expressed the transgene. The relative absence of *lacZ* expression in basal cells was demonstrated in grafts infected with either Ad.RSVβgal or Ad.CMV*lacZ*, suggesting that variation of the viral promoters driving transgene expression is not the cause for the exclusion of β-galactosidase activity found in basal cells.

Diffusion of Xgal precipitate made the quantification of cell types within large, highly expressing clusters difficult. A more precise definition of the cell types expressing the transgene was achieved by performing immunocytochemistry with antibodies to the reporter gene product β-galactosidase and to one specific for either basal cells (cytokeratin 14) or differentiated columnar cells (cytokeratin 18). Frozen sections (6μm) from xenografts infected with $1x10^{11}$ pfu/ml Ad.CMV*lacZ* and harvested 3 days post-infection were analyzed by triple immunofluorescence (Nomarski) with antibodies to: β-galactosidase (conjugated to Texas Red); cytokeratin 14 (conjugated to AMCA); and cytokeratin 18 (conjugated to FITC). *LacZ* co-localized with the differentiated cell marker cytokeratin 18 in > 99.9% transgene expressing cells (N=1500 cells counted) from both Ad.CMV*lacZ* and Ad.RSVβgal infected grafts, thereby confirming the observations made from Xgal stained grafts.

Additional experiments were performed with recombinant adenoviruses expressing human CFTR to establish the validity of this model for developing gene therapies for cystic fibrosis. Frozen sections (6μm) from xenografts infected with either $1x10^{11}$ pfu/ml of Ad.CB-CFTR or Ad.CMV*lacZ* and harvested 3 days post-infection were analyzed by *in situ* hybridization using a human CFTR R-domain probe, and by immunocytochemistry for CFTR protein using a polyclonal CFTR antibody. Engelhardt, J.F. et al., *Nature Genet.* 2:240-248 (1992). Hybridization above background was detected with the antisense CFTR probe in approximately 2-10% of cells in Ad.CB-CFTR infected grafts. A similar proportion of cells in these grafts demonstrated over-expression of CFTR protein based on immunocytochemistry with a CFTR specific antibody. Ad.CMV*lacZ* infected xenografts failed to demonstrate hybridization to the CFTR probe or binding to the CFTR antibody that was above endogenous levels. Over-expression of CFTR protein in Ad.CB-CFTR infected grafts was detected in all differentiated cell types including ciliated cells, goblet cells, and intermediate cells. The recombinant protein localized to the apical surface in most of the ciliated and goblet cells and to the cytosol of intermediate cells. Expression of recombinant CFTR protein was detected in grafts for at least 5 weeks after infection.

Expression of adenoviral proteins in epithelial cells of genetically reconstituted xenografts. Immunocytochemical techniques were used to analyze xenografts for expression of adenoviral proteins. Antibodies that recognize hexon, fiber, and the 72 kd E2a gene product, *i.e.* DNA binding protein (DBP), were used to detect adenoviral protein expression in xenografts infected with Ad.CMV*lacZ*,

Ad.RSVβgal, or wild type Ad5. Frozen sections (6 μm) of xenografts infected with $1x10^{11}$ pfu/ml Ad.CMV*lacZ* and harvested 3 days post-infection were analyzed by triple immunofluorescence (Nomarski) with antibodies to β-galactosidase (conjugated to AMCA); DBP (conjugated to Texas Red); and fiber (conjugated to FITC). Xenografts infected with wild type Ad5, harvested 20 hr post-infection and analyzed by double immunofluorescence (Nomarski) with antibodies revealed a subpopulation of cells that expressed high levels of hexon and fiber protein. Double immunofluorescence studies indicated that cells expressing the late gene products hexon and fiber also expressed low levels of the gene product DBP, which is expressed in the early phase of adenovirus infection. Similar analyses of xenografts infected with $1x10^{10}$, 10^{11} and 10^{12} pfu/ml of Ad.RSVβgal or Ad.CMV*lacZ* failed to demonstrate detectable levels of either fiber or hexon proteins despite substantial levels of β-galactosidase expression. However, with longer incubation times and higher concentrations of primary antibody low levels of fiber expression could be seen in nuclei of a few *lacZ* expressing cells. In contrast, high levels of DBP were found in 3-5% of β-galactosidase positive cells with an occasional cell expressing DBP in the absence of detectable β-galactosidase. Cells expressing DBP tended to express lower levels of β-galactosidase and were predominantly found in clusters. The percentage of DBP expressing β-galactosidase positive cells was the same for MOI's of $1x10^{10}$, 10^{10} and 10^{12} pfu/ml.

Recovery of adenovirus from xenografts. Xenografts were subjected to sequential irrigations for a period of up to 24 days after infection. Effluents (1 ml) were collected at 3 1/2-day intervals from xenografts infected with Ad.CMV*lacZ* and were titered by Xgal-stained pfu assay on 293 cells. All plaques generated on 293 cells contained β–galactosidase as was evident by the formation of a blue precipitate. Recovered virus was plotted on a log scale versus the time after infusion of virus, measured in days. Figure 2(A-E) presents representative experiments. The concentration of virus in the effluents dropped precipitously during the initial week following infection in all grafts except that presented in Figure 2B, in which virus transiently increased before the exponential decline. The amount of virus recovered in the effluents stabilized at low but detectable levels during the remaining period of observation (days 14 to 24) in most but not all of the grafts.

Following the completion of the experiment, the xenografts were harvested, Xgal-stained and evaluated for percentage genetic reconstitution in the surface epithelial cells. The quantity of virus recovered in effluents at the end of the

experiment varied substantially between grafts and was proportional to the percentage of the epithelium that expresses the transgene: in Figure 2(A-E), panels A-C, 5-20% *lacZ* positive cells; panel D, 1% *lacZ* positive cells; and panel E, less than 0.01% *lacZ* positive cells. The highest concentration of virus was detected in effluents from the xenograft with the greatest level of genetic reconstitution (Figure 2A) whereas the graft that produced effluents with no detectable virus at 24 days was found to have very little transgene expression in its epithelium (Figure 2E). All viruses recovered in the effluents were found to express *lacZ*, indicating that the xenografts were not grossly shedding wild type adenovirus.

To further address the possibility of wild type adenoviral contamination in the tissue samples or recombinant stocks, 100 µl of selected 1 ml effluents were used to infect 80% confluent layers of HeLa cells. In Figure 2, asterisks mark effluents that were assayed for wild type adenovirus by the ability to cause cytopathic effects (CPE) on HeLa cells. Following infection, the media was changed every 48 hr for the first 4 days and every day for the following 17 days. No evidence of CPE was seen with any of the effluents. Finally, polymerase chain reaction (PCR) analysis of effluents failed to detect E1a sequences at a sensitivity of 100 molecules per ml effluent.

SPECIFIC EXAMPLE 2 - Temperature-sensitive Vectors

Vectors with the CFTR minigene inserted into the E1-deleted region of sub360 and dl7001 backbones, also containing additional mutations in genes necessary for viral replication, have been produced. As further discussed below, the incorporation of the missense temperature-sensitive mutation found in the H5ts125 strain at 62.5 mu, is achieved by combining fragments from three independent DNA constructs including sub360 or dl7001, H5ts125, and a CFTR cDNA plasmid with E1a sequences placed 5' to the minigene cassette. These vectors are designated Ad.CB-CFTRts125sub360 and Ad.CB-CFTRts125dl7001. These vectors are identical except for the size of deleted regions in E3. Figure 3 shows the location of the CFTR problem transgene within the E1-deleted region, E3-deletion, and the H5ts125 mutation with respect to other adenoviral genes. Due to the mutations in these vectors, there is reduced viral replication, reduction in expressed protein and an increase in the persistence of transgene expression. The following is a more detailed description of the production of the vectors of the present invention.

Temperature-sensitive vectors. In constructing the temperature-sensitive vectors of the present invention, temperature-sensitive (*ts*) adenovirus stocks, which

served as "parental" stocks of vectors of the present invention, were generated. These vectors have *ts* mutations in the (DBP)E2a region. Specifically, a previously isolated temperature-sensitive mutant Hst125 of the adenovirus type 5 strain has been obtained. Vander Vliet, P. et al., *J. Virol.* 15:348-354 (1975). Hst125 has a single amino acid substitution (62.5 mu) at the carboxy end of the 72kd protein produced from the E2a gene. This protein product is a single-stranded DNA binding protein and is involved in the replication of adenoviral genomic DNA. At permissive temperatures (approximately 32°C) this strain is capable of full life cycle growth on HeLa cells, while at non-permissive temperatures (approximately 38°C) no replication of adenoviral DNA is seen. In addition, at non-permissive temperatures, decreased immunoreactive 72kd protein is seen in HeLa cells.

Two double mutant stocks have also been generated which contain the Hst125 mutation and E3 deletions. The E3 mutations are characteristic of the previously described mutant adenovirus type 5 strains, sub360 and dl7001. (sub360 published in Logan, J. et al., *Proc. Natl. Acad. Sci. (USA)* 81:3655-3659 (1984); dl7001 (kindly provided by Dr. William Wold, Washington University, St Louis)). Both mutant viruses contain a deletion in the E3 region of the adenoviral genome; in sub360, at 78.5 to 84.3 mu, and in dl7001, at 78.4 to 86 mu. The life cycle of both sub360 and dl7001 display wild type characteristics, as E3 is not required for replication of adenovirus.

The resultant new adenovirus mutants Hst125sub360 and Hst126d17001 are used to generate CFTR recombinant adenovirus vectors which carry the Hts125 mutation and E3 deletion, wherein CFTR is inserted into the E1a/E1b-deleted regions. The CFTR gene cassette is driven by the CMV enhancer and β-actin promoter. As stated above, the resultant recombinant adenovirus vectors are designated Ad5.CB-CFTRst125sub360 and Ad5.CB-CFTRst125dl7001.

Temperature-sensitive CFTR vectors. The following is a more detailed description of the generation of the CFTR recombinant adenovirus of the present invention. The plasmid Pad.CB-CFTR was linearized by Nhe I cleavage. 293 cells were cotransfected with Ad.CB-CFTR and the large fragment of Cla I-cut Hst125dl7001 or Hst125sub360 DNA, to allow homologous recombination to occur. The recombinant adenoviral DNA was replicated and encapsidated into infectious virions, as evidenced by formation of plaques. Virus was isolated from individual plaques following incubation at 32°C, and amplified in 293 cells. As expected, no viral plaques were seen at 38°C, the nonpermissive temperature. Recombinant

adenoviruses containing the human CFTR cDNA were identified by restriction cleavage and Southern blot analysis. Two of the recombinant viruses positive for CFTR were plaque-purified a second time, and designated Ad.CB-CFTRts125dl7001 and Ad.CB-CFTRts125sub360. These viruses were propagated in 293 cells by infection at 32°C. After 56 hr, viruses were recovered by 3 cycles of freeze thawing. All viral preparations were purified by CsCl density centrifugation and either followed by gel filtration to remove CsCl for immediate use, or stored at -20°C after a 1:5 dilution into a glycerol/BSA solution. Titers of viral stocks were determined by plaque assay using 293 cells.

As those skilled in the art will realize, related adenoviral serotypes such as adenovirus type 2, as well as any of the forty-one adenoviral serotypes, may be substituted for the adenoviral type 5 vectors described in the present invention. Thus, it is not Applicants' intention to be bound by a specific adenoviral type.

SPECIFIC EXAMPLE 3 - Primate Studies

The primate studies have attempted to simulate the clinical trials in order to assess toxicity and biological efficacy with first generation vectors. The results are tabulated in Tables I-X, which are set forth at the end of this Specific Example. Although transgene (CFTR) expression was achieved, side effects of the first generation vectors included lung tissue inflammation. One aspect of the preferred second generation vectors of the invention is to ameliorate such inflammation.

A. STUDY I - Feasibility

The goal of this study was to assess the feasibility of selectively delivering recombinant adenovirus to a single segment of a dependent lobe. The highest dose of virus proposed in the clinical trials was used in this experiment. A *lacZ* virus of similar structure to the Ad.CB-CFTR was used so that the distribution of gene expression in the whole lung could be accurately evaluated using sensitive and specific histochemical stains. More specifically, the *lacZ* transgene cassette is substituted for the CFTR transgene; all adenoviral sequences are the same. Specific goals were to assess short-term toxicity and to evaluate the distribution of recombinant gene expression within and beyond the targeted pulmonary segment.

Materials and Methods

Animal. A 12-year old, 32.5 kg baboon (*Papio anubis*) was used for this study (CFB1). During pre-transfection evaluation, it was found that the animal was hypoxemic (PaO_2 54 mmHg) and hypercarbic ($PaCO_2$ 55 mmHg). Chest X-ray

showed that his right upper and right middle lobes were collapsed. It was decided that he was unsuitable as a long-term animal and therefore was used for a short-term feasibility study. Subsequent experience led to the conclusion that the majority of the abnormalities in gas exchange were due to atelectasis that occurred during anesthesia, rather than intrinsic lung disease.

Anesthesia. The animal was sedated with intramuscular tiletamine/zolazepam (Telazol) (2.2 mg/kg). Repeated injections were used to maintain adequate sedation. An intravenous catheter was inserted into a saphenous vein and Ringer's lactate infused during the procedure. The animal breathed room air during the procedure.

Virus administration. The animal was intubated with a 9 mm cuffed endotracheal tube by the oral route. Benzocaine (20%) was sprayed into the endotracheal tube. An Olympus BF 1T20 flexible fiberoptic bronchoscope was introduced into the airway. Inspection of the airway revealed no proximal obstructing lesion which would explain the right upper and right middle lobe collapse seen on chest X-ray. The bronchial mucosa was slightly friable throughout the tracheobronchial tree. A 5 Fr double lumen balloon catheter was inserted into the left lung through the bronchoscope channel. The tip was guided into the orifice of the posterior segment of the left upper lobe and the balloon was inflated with 0.8 ml of air. Twenty-five ml of Ad.CMV-*lacZ* virus in normal saline ($1x10^{11}$ pfu/ml) was injected through the catheter into the cannulated segment. Five ml of air was injected to clear the catheter of virus-containing fluid. The animal did not cough and no liquid was seen to leak from the bronchial orifice during the infusion. The catheter with balloon inflated was held in place for 8 minutes. At the end of that time, palpation of the animal's pulse detected several premature beats. The catheter and scope were removed. The animal was kept in the supine position for another 10 minutes. During this time, the animal was ventilated with an ambu-bag because 4 - 5 premature beats/minute were noted. The animal did not cough during this period. Within 10 minutes, the premature beats were no longer detected and the animal was returned to his cage where he recovered uneventfully.

Follow-up: Necropsy. The animal was sedated with tiletamine/zolazepam (Telazol) (2.2 mg/kg) and sacrificed with pentobarbital/phenytoin. A necropsy was performed. The lung was inflation fixed and stained *en bloc* in Xgal.

Results

The animal tolerated the procedure well without clinical evidence of toxicity. Necropsy was performed with full gross and histopathology.

Three days post-infection, the baboon was euthanized and the main stem bronchi to the left lung was ligated to tubing. The baboon's two lungs were inflation fixed in 0.5% glutaraldehyde/PBS for 2 hr followed by rinsing in 1mM $MgCl_2$/PBS two times for 15 min each. The lung was then stained *en bloc* in Xgal solution for 30 min at 37°C by filling the lung with reaction mixture in addition to immersing the lung in the same buffer. The Xgal solution was then removed and the lungs were postfixed in buffered formulin. The reaction proceeded for two hours. Gross inspection of the left lung revealed intense Xgal reaction product apparently confined to the posterior segment of the left upper lobe. As shown in Figure 4, a small focus of reaction product was seen in the left lower lobe. In Figure 4, the solid arrowhead points to the Xgal reaction product in the segment infused with virus, while the open arrowhead marks an area in which virus leaked into an adjacent segment of a neighboring lobe. Histological sections revealed recombinant gene expression primarily in alveolar cells and patchy but sparse expression in conducting airway, similar to what was seen in Xgal stained tissue from CFTR and *lacZ* infused lobes of a CFB4 animal shown in Figures 5 and 6. (The protocol for the CFB4 animal was as follows: the most proximal segment of the right upper and left upper lobes of the CFB4 animal was infused with seven ml of Ad.CB-CFTR and Ad.CMV*lacZ* at a concentration of 1 x 10^{10}pfu/ml. Samples from quadrant 1D were fixed in 0.5% glutaraldehyde/PBS and stained on block for β-galactosidase with Xgal for 30 min. Tissue was embedded in paraffin, sectioned at 5μm, and stained briefly in hematoxylin). The photomicrographs of Figure 5 show *on fos* views from a dissecting microscope from the Ad.CB-CFTR infected lobe (Figure 5A) and the Ad.CMV*lacZ* infected lobe (Figure 5B). The black arrow points to Xgal positive cells in bronchus, while the white arrow points to Xgal positive regions in the alveoli. The photomicrographs of Figure 6 show sections from the Ad.CB-CFTR infected lobe (Figure 6A) and the AD.CMV*lacZ* infected lobe (Figure 6B). On gross examination, the lungs appeared normal. Atelectasis complicated interpretation of the histologic sections. However, focal areas of alveolitis with nonsuppurative perivascular mononuclear cell infiltrates were seen confined to the areas of the lung in which the gene was instilled (discussed in more detail below).

B. STUDY II - Toxicity

The goal of this study was to assess the long term toxicity of recombinant adenovirus delivered to pulmonary segments. In addition, an attempt to evaluate the sensitivity and specificity of bronchoscopic techniques for detecting recombinant gene expression and adenoviral infection was made. A large baboon was administered maximal doses ($1x10^{10}$ pfu/ml) of Ad.CMV-*lacZ* and Ad.CB-CFTR to individual pulmonary segments. The animal has been studied with several bronchoscopies with bronchoalveolar lavage and brushings, with specimens analyzed for pathology as well as *lacZ* and CFTR expressing cells.

Material and Methods

Animal. A 12-year old, 32.5 kg male baboon (*Papio cynocephalus/anubis*) was used for these studies (CFB2). He was maintained in a cage separate from other animals and fed a standard diet.

Anesthesia. The animal was sedated with intramuscular tiletamine/zolazepam (Telazol) (2.2 mg/kg). Sedation was maintained by supplemental injections with the same agent and with intramuscular butorphanol (0.02 mg/kg). An intravenous catheter was inserted into a saphenous vein and Ringer's lactate was infused during the procedure. On follow-up study days, the animal was initially sedated with intramuscular tiletamine/zolazepam, but supplemental sedation was accomplished with intravenous thiamylal (2.5 mg/kg) as needed.

Chest X-rays and specimen collection. Ventral-dorsal and left lateral recumbent chest X-rays were performed. Blood was drawn from the femoral artery and analyzed for arterial blood gases, chemistries, hematologic measurements, coagulation parameters, and viral cultures (see below). A urethral catheter was inserted into the urinary bladder and urine was obtained for routine analysis and viral culture. Cotton swabs were used to obtain rectal and pharyngeal samples for viral culture.

Bronchoscopic sampling. A 9 mm cuffed endotracheal tube was inserted into the trachea by the oral route. A FB-18X Pentax fiberoptic bronchoscope was passed into the trachea and advanced to a wedged position in the right middle lobe. Twenty five ml of sterile saline was instilled and immediately aspirated yielding 12 ml for analysis. Next, 3 bronchial brushes were used to obtain epithelial cells from the right middle lobe bronchus. Each brush was advanced approximately 3 cm into the lobar orifice and rubbed back and forth. The distal 4 cm of the brushes were cut off and dropped into Ham's F12 medium.

Virus administration. The fiberoptic bronchoscope was inserted into the trachea and advanced to the orifice of the posterior segment of the left upper lobe. A 5 Fr. double lumen balloon catheter was passed through the channel of the bronchoscope and advanced so that its balloon was just within the orifice of the segmental bronchus. The balloon was gently inflated to occlude the bronchus. Twenty ml of Ad.CMV-*LacZ* virus at $1x10^{10}$ pfu in normal saline was infused into the segmental bronchus through the catheter lumen. The catheter was kept in place for 10 minutes and then removed. Next the bronchoscope was directed to the segmental orifice of the posterior segment of the right upper lobe. As on the left side, a balloon catheter was used to instill 20 ml of Ad.CB-CFTR into the segmental bronchus. Prior to and after the infusion of virus, the animal had apneic episodes, presumably from the accumulated effects of repeated intramuscular sedation. Intermittently during and after the infusion, the animal was ventilated by Ambu bag until spontaneous respirations became steady. Naloxone and doxepram were also given. The animal was returned to his cage and recovered uneventfully.

Follow-up studies. On days 3, 14, 21, 73, 77, 79, 93, 102, 116 and 152 the animal was anesthetized. Chest X-rays were obtained, and blood, urine, stool, and pharyngeal specimens were taken. On days 3 and 21, the trachea was intubated and the bronchoscope used to obtained bronchoalveolar lavage fluid and bronchial brush specimens from the posterior segment of the right and left upper lobes as outlined above.

Results

The animal tolerated the procedure well and experienced no obvious clinical toxicity. The animal continues to gain weight and its vital signs remain within normal limits. The laboratory results from the multiple studies performed were recorded together with the results of Study III in Tables II-X and Figures 8-12. To summarize the results for CFB2, the blood hematologies (Table II), coagulation profile (Table II), chemistries (Table III), and urinalyses (Table IV) have remained within normal limits with the exception of hepatic enzyme levels (Table III). Mild elevations of transaminase were seen prior to gene transfer. The elevated levels have either persisted or improved during the follow-up period. Arterial blood gases (Table V) showed baseline hypoxemia and hypercarbia (discussed below). To monitor for changes in gas exchange during the follow-up period, the $P(A\text{-}a)O_2$ was used. There was an increase in $P(A\text{-}a)O_2$ that peaked on day 21 and decreased to below baseline on day 116 post gene administration. Chest X-rays (Table VI) showed mild

upper lobe haziness prior to gene administration. The abnormalities worsened by day 21 on the left side. Thereafter, the chest radiographs returned to normal.

Analysis of BAL fluid cell number and differential (Table VII) is discussed in conjunction with the Dose/Toxicity Study III, below. As shown in the photomicrograph of Figure 7A, histochemical Xgal staining of cytocentrifuged preparations of bronchial brushing, from the Ad.CMV*lacZ* infused left upper-lobe of the CFB4 aminal, showed *lacZ* expression in 5.2% of cells in BAL fluid that were recovered from the *lacZ* segment on day 4; a single *lacZ* cell was recovered on the contralateral side. CFTR expression was detected in 3.4% of cells in BAL fluid from the CFTR segment (see Figure 7B). As shown in the photomicrograph of Figure 7B, immunocytochemical CFTR staining of cytocentrifuged preparations of bronchial brushings from the Ad.CB-CFTR infused right upper lobe of the CFB4 animal showed CFTR in 2.1% of the cells that received the Ad.CB-CFTR (Table VIII). No transgene expression was detected in cells recovered from brushings or BAL beyond 4 days after infusion.

The results of culture for viral shedding and recovery is presented with the results from the Dose/Toxicity Study III below, (Tables IX and X).

C. STUDY III - Dose/Toxicity Study

This study used 12 baboons to determine the efficacy and toxicity of adenovirus-mediated gene transfer (Table I). Each of the 12 animals received intrabronchial instillation of Ad.CMV-*lacZ* into the posterior segment of the left upper lobe and Ad.CB-CFTR into the posterior segment of the right upper lobe. The animals were divided into four groups of 3 animals, with each group receiving a different concentration of virus in the fluid instilled into the bronchi: $1x10^7$, 10^8, 10^9, or 10^{10} pfu/ml. One member of each of the four groups was necropsied on day 4 following gene infusion, another on day 21, and the remaining animal was kept alive for long-term evaluation.

Materials and Methods

Animals. The 12 baboons (*Papio*) used for the main dose-response study were 2 to 5-year old and weighed 7 to 14 kg (Table I). All animals were caged separately and fed standard diets.

Sedation. After allowing no oral intake for 12 hours, the animals were sedated by intramuscular injection of 2.2 mg/kg tiletamine/zolazepam (Telazol). Adequate sedation was maintained either with repeated intramuscular doses of the same agents or with intravenous thiamylal (2.5 mg/kg).

Specimen collection and chest radiographs. After sedation, arterial blood was drawn from the femoral artery into a heparinized syringe for measurement of blood gases, and aliquots were placed into sodium EDTA for measurement of blood counts, into glass tubes for measurements of serum chemistries and antibodies, into a heparinized tube for viral culture, and into sodium citrate for measurement of prothrombin and partial thromboplastin times. A urethral catheter was inserted into the bladder to obtain urine for routine analysis and viral culture. The clinical laboratories of University Hospital, Ann Arbor, MI were used to analyze the blood for cell counts, differential counts, coagulation measurements, and arterial blood gases; the serum for electrolytes, blood urea nitrogen and creatinine, calcium, phosphate, total protein, albumin, aspartate and alanine transaminase, alkaline phosphatase, lactic dehydrogenase, and bilirubin; and the urine specimens for routine urinalysis. Nasopharyngeal secretions and rectal stool samples were collected by cotton swabs. These samples together with blood, urine, and bronchoalveolar lavage fluid were cultured for adenoviruses. Ventral-dorsal and left lateral recumbent chest radiographs were performed.

Bronchoscopy. A FB-10X Pentax fiberoptic bronchoscope was inserted through a 5.5 mm uncuffed endotracheal tube. Bronchoalveolar lavage was performed by instilling normal saline (10 ml for the smaller animals) through a wedged bronchoscope as a single bolus. The fluid was immediately aspirated and placed onto ice. Bronchial brushings were performed using 3-4 cytology brushes (Olympus BC-12C) for each segment sampled. The brushes were advanced approximately 2-3 cm beyond to the lobar orifice, rubbed back and forth, and then removed. The distal 4 cm of the brushes were cut off and dropped into Ham's F12 medium.

Study protocol. After sedation and intratracheal intubation, bronchoalveolar lavage fluid and bronchial brushings were obtained from the right middle lobe. Ad.CMV-*lacZ* in 1% glycerol/normal saline was infused into the posterior segment of the left upper lobe and Ad.CB-CFTR in 1% glycerol/normal saline into the posterior segment of the right upper lobe. Unlike the procedure used for the two larger pilot animals, the balloon catheter could not be used in the smaller 12 animals because of limitations in size of the channel in the smaller bronchoscope. Instead, the virus was infused (7 ml of $1x10^7$, 10^8, 10^9 or 10^{10} pfu/ml) through the internal channel of the bronchoscope with the bronchoscope wedged into the bronchial orifice. After viral instillation, the bronchoscope was kept wedged for 10 minutes.

The bronchoscope was removed and the animal maintained in the supine position for a minimum of 10 more minutes. This technique appeared less satisfactory than using the balloon catheter as was done in the larger animals, because complete occlusion of the airway was difficult to maintain during and after viral infusion.

Follow-up: Necropsy. One animal from each group was sacrificed on day 4 and another on day 21. Immediately before sacrifice, the animal was anesthetized as outlined above, chest X-rays were performed, and clinical specimens including blood, urine, stool and pharyngeal secretions were taken. The animal was then sacrificed with pentobarbital/phenytoin (Beuthanasia) and a necropsy was performed.

Follow-up studies: Long-term animals. One animal from each group was kept alive for repeated evaluations. On days 4, 15, and 21, the animals were anesthetized, chest X-rays were obtained, and blood, urine, stool and pharyngeal specimens were taken. On days 4 and 21, the trachea was intubated and the bronchoscope used to obtained bronchoalveolar lavage fluid and bronchial brush specimens from the posterior segments of the right and left upper lobes as outlined above. Additional blood samples and chest radiographs were obtained on at least a monthly basis for the long-term animals.

Concurrent studies. Animals CFB3, CFB4, and CFB6 also participated in a study of adenovirus-mediated gene transfer into the nasal mucosa. Immediately prior to intrabronchial instillation of adenovirus as outlined above, the animals were placed into the right lateral recumbent position. A polyethylene catheter was inserted 4-5 cm into each nose and 0.8 ml of virus was infused over a 15 minute period into the right nostril and 0.8 ml vehicle (3.3% glycerol/33% PBS/63.7% normal saline) alone into the left. CFB3 and CFB4 received Ad.CB-CFTR at $1x10^7$ and 10^{10} pfu/ml, respectively; CFB6 received Ad.CMV-*LacZ* at 1×10^{10} pfu/ml.

General response of animals. The animals tolerated the bronchoscopic instillation of Ad.CB-CFTR and Ad.CMV-*LacZ* without complication except that 3 animals vomited immediately after intratracheal intubation prior to gene infusion. Two of these animals also vomited when intubated during the follow-up bronchoscopies. In none of these episodes was intratracheal aspiration observed bronchoscopically. During the post-transfection period, the behavior of the animals was normal. They continued to eat normally and their weight changed less than 3% in the post-transfection period. Rectal temperatures when measured while the animals were sedated for studies were never elevated.

Hematologic counts and coagulation measurements. Blood was drawn into tubes containing sodium citrate from the femoral artery immediately prior to intrabronchial gene administration (day 0) and again on days 4, 15 and 21. As shown in Figure 8A, blood hemoglobin concentrations remained within normal range in all animals during the 3 weeks following transfection. As shown in Figure 8B, when the animals were analyzed as a single group, white blood cell counts decreased between day 0 and day 4 ($p<0.01$), but remained within the normal range. The extent of reduction was not directly related to viral dose. After day 4, the counts increased so that by day 15 they were no different from baseline ($p>0.1$). White blood cell differential counts were normal except that at some time during the study, 6 animals had mildly elevated percent monocytes (maximum of 11%). Three of these animals had monocyte elevations on day 0 prior to gene administration. The other 3 animals belong to groups that received $1x10^7$, 10^8, and 10^9 pfu/ml doses. As shown in Figure 8C, blood platelet counts (expressed as the mean $\pm$ SEM of all animals tested) remained in the normal range on all measurements and did not change during the study. The prothrombin time and partial thromboplastin time remained within the normal range for during the study. Also, see Table II.

Serum electrolytes, proteins, enzymes and urinalyses. Serum concentrations of Na^+, K^+, and Cl^- were normal and remained unchanged throughout the study. See Table III. Two animals had low serum HCO_3^- levels on day 0 (15 and 17 mEq/L) prior to gene administration. Arterial blood pH was also low in these samples (discussed below). During the follow-up period, the HCO_3^- increased toward the normal range. Calcium, phosphorous, total protein and albumin remained normal. Six animals had low levels of proteinuria on one or more urine samples during the study. See Table IV. Several of these were likely due to traumatic catheterizations since gross hematuria was also present in the catheterization specimen but not noted when the animals spontaneously voided later in the day. The proteinuria was not related to viral dose or the number of days post-transfection. Serum creatinine and blood urea nitrogen were normal in all animals throughout the study. Liver function tests (aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase, alkaline phosphatase, and bilirubin were normal except for one animal that had mild enzyme elevations at baseline (*e.g.* AST 56 IU/L, ALT 85 IU/L) that remained stable or improved during the study. Prothrombin time and partial thromboplastin time were always normal.

Arterial blood gases. Arterial blood samples obtained while the animals were sedated prior to gene administration showed a moderately wide range of $PaCO_2$ levels with a mean ± SD of 44.8 ± 4.3 mmHg (range 36.7 to 54.7 mmHg). See Table V. The elevated $PaCO_2$ levels are likely due to hypoventilation and atelectasis which has been previously reported when baboon are sedated. The measured levels of $PaCO_2$ remained elevated throughout the study with no changes directly related to viral dose or to the number of days post-transfection.

As mentioned above, arterial blood pH levels were low in two animals at baseline prior to gene transfection (pH 7.15 and 7.28). The $PaCO_2$ levels were no higher in these two animals, so acute hypoventilation was not solely responsible. The anion gap ($Na^+ - [K^+ + HCO_3^-]$) was increased indicating a metabolic acidosis. The unmeasured anions were not identified, but were unlikely to be ketone bodies because none were detected in the urine. During the study, the low pH levels improved in these animals; the pH of one animal increased from 7.15 to 7.32 by necropsy on day 4, and of the other animal from 7.28 to 7.39 by day 15.

Baseline PaO_2 was 78.1 ± 11.0 mmHg (mean ± SD, range 54.0 to 98.0 mmHg). To compensate for changes in PaO_2 caused by changes in ventilation, the PaO_2 data were analyzed using the calculated alveolar to arterial oxygen gradient ($P(A\text{-}a)O_2$) using measured arterial PaO_2 and $PaCO_2$. Blood was drawn from the femoral artery immediately prior to intrabronchial gene administration (day 0) and again on days 4, 15 and 21. Additional samples were obtained on later days from the long-term animals receiving 1×10^{10} pfu/ml viral dose. The mean $P(A\text{-}a)O_2$ on day 0 prior to gene administration was 12.5 ± 7.0 (SD) with a range of 0.0 to 26.6. Because the wide range of baseline $P(A\text{-}a)O_2$ levels might obscure a treatment-induced change in gas exchange, the data were analyzed using the change in $P(A\text{-}a)O_2$ from the day 0 level ($\Delta P(A\text{-}a)O_2$). When analyzed in this fashion, no statistically significant effect on $\Delta P(A\text{-}a)O_2$ was found for viral dose or from the time interval post-transfection. However, inspection of the relationship between $\Delta P(A\text{-}a)O_2$ and the number of days post-transfection did suggest a trend. Figure 9 shows the change in $P(A\text{-}a)O_2$ in mmHg from the level on day 0 ($\Delta P(A\text{-}a)O_2$) for groups of animals receiving each dose of virus. The higher dose animals ($1x10^9$ and 10^{10} pfu/ml) were more likely to have an increase in $P(A\text{-}a)O_2$ while the lower dose animals ($1x10^7$ and 10^8 pfu/ml) were more equally distributed between increased and decreased $P(A\text{-}a)O_2$ after gene administration.

Chest radiographs. Supine ventral-dorsal chest radiographs were taken in all animals. See Table VI. Detection of infiltrates was complicated by transient atelectasis that apparently occurred when the animals were sedated. It was noted that the side of the animal on which the atelectasis occurred was often the side on which the animal was lying prior to taking the X-ray. Repositioning the animal into the opposite decubitus position for a minute and then rotating him to the supine position would expand the atelectatic area. After compensating of these artifacts, it was then possible to analyze the chest radiographs for appearance of abnormalities. No infiltrates appeared in any animal receiving $1x10^7$, 10^8, or 10^9 pfu/ml doses. New alveolar infiltrates occurred only in 3 of the 4 animals receiving $1x10^{10}$ pfu/ml dose. To localize and quantify the extent of infiltrate, a grading system was used. The upper, middle, and lower lung fields of each lung were graded for alveolar infiltrates: "mild" - minimally detectable infiltrate; "moderate" - infiltrate occupying 1/4 of lung field; "severe" - infiltrate occupying 1/2 or more of lung field. Figure 10 shows the location and extent of chest radiographic abnormalties detected before and after gene administration in animals receiving a 1×10^{10}pfu/ml dose. As shown in Figure 10, the infiltrates generally first appeared on days 15 and 21 post-transfection. In one of the long-term animals, streaky infiltrates were present in both upper lobes on day 0 prior to transfection. The infiltrate worsened on day 21, but completely resolved by the radiograph taken on day 73. The infiltrate that appeared in the other long-term, 1×10^{10} pfu/ml animal completely resolved by the X-ray performed on day 39.

Results

Bronchoscopic results. Bronchoalveolar lavages was performed on long-term animals 3 times during the study and the results are shown in Figure 11 and Table VII. On day 0, the right middle lobe was lavaged (solid symbols in Figure 11), and on days 4 and 21 the posterior segments of right (solid symbols in Figure 11) and left (open symbols in Figure 11) upper lobes were lavaged. Cytocentrifuge preparations were made and the cells stained with a modified Wright's stain. In Figure 11, data from animals receiving 1×10^7 (□, ■), 10^8 (△, ▲), 10^9 (▽, ▼), or 10^{10} (○, •)pfu/ml are displayed separately. Approximately 50% of the instilled volume was recovered with no variation depending on viral dose or time following gene administration. The cell concentration of the bronchoalveolar lavage fluid obtained from the right middle lobe on the day of gene instillation was $2.32 \times 10^6 \pm 1.08 \times 10^6$ (SD) cells/ml. Cell numbers measured in lavage fluid from the follow-up

bronchoscopies showed no relationship to day, dose, or side on which the lavage was performed. Analysis of the cell differential counts was complicated by the finding that on day 0, one animal had markedly elevated percent neutrophils and another had elevated percent lymphocytes. Even with these suspected outliers, the percent neutrophils when analyzed for all animals changed significantly over time ($p<0.03$) with a peak at 4 days and a reduction back to baseline at day 21. (See Figure 11). This pattern occurred independent of the dose of virus administered. The percent lymphocytes also changed significantly over time ($p<0.04$) with an increase occurring later than the neutrophils, between day 4 and day 21. This increase was not influenced by the dose of virus administered and was independent of the side lavaged. Sporadic increases were seen in percent eosinophils; often the eosinophils were present in only one of the two segments lavaged (not consistently the left or right side) and then only for a single point in time.

Cytocentrifuge smears were stained with Xgal to detect β-galactosidase activity (Table VII). Blue-staining cells were present within the cell populations obtained from animals receiving $1x10^{10}$ pfu/ml doses at the day 3 or 4 time point. No cells stained positively for β-galactosidase from fluids obtained at other times or from animals receiving lower concentrations of virus. The cells obtained from one 1×10^{10} pfu/ml dose animal were stained for CFTR using immunocytochemistry. Small numbers of cells (3.4%) stained positively on day 3 after gene administration.

Bronchial brushings. Cytocentrifuge smears of cells obtained by bronchial brushings were analyzed for transgene expression by staining for β-galactosidase with Xgal and for CFTR by immunocytochemistry. See Table VIII and Figure 7A and 7B. Small numbers of cells stained positively in samples obtained from animals 3 or day 4 after receiving $1x10^{10}$ pfu/ml doses.

Viral cultures. Specimens from blood, urine, nasopharynx, stool and bronchoalveolar lavage fluid were cultured for the presence of Ad.CMV-*lacZ* (by Xgal staining of 293 cell monolayers) and for adenoviruses by examining 293 monolayers for cytopathic effects. See Tables IX and X. Positive control data are also presented. All cultures yielded no growth except for one culture of bronchoalveolar lavage fluid obtained on day 3 after gene administration from an animal that received $1x10^{10}$ pfu/ml dose.

Necropsy. Grossly, the lungs of all animals appeared normal with two exceptions. The animal that was sacrificed 21 days after receiving $1x10^{10}$ pfu/ml virus had hemorrhagic and grayish patches located predominantly over the dorsal

surfaces of the right upper, left upper and right lower lobes. Also, one animal had punctate green-black colored 1 mm spots scattered over the entire surface of both lungs.

Expression of β-galactosidase activity was examined using lung sections stained *en bloc* with Xgal. Alveolar tissue from the posterior segment of the left upper lobe of animals receiving $1x10^{10}$ pfu/ml doses stained dark blue on day 3 or 4 (see Figure 5). The animal receiving $1x10^{9}$ pfu/ml had similar, but less intense staining on day 4. A few scattered areas of positively staining airway cells could be seen under the dissecting microscope from the animals receiving $1x10^{10}$ pfu/ml on day 3 or 4. No staining was seen in any animal on day 21 or at lower viral doses. Xgal staining of frozen sections of lung tissue confirmed the conclusions made at the dissecting microscope level. (See Figure 6).

Southern analysis was performed to determine the anatomical location, copy number and persistence of viral DNA. Total DNA was prepared from adjacent quadrants of the lung which were analyzed for transgene expression by histochemical and immunohistochemical techniques. In Figure 12, each field (upper, middle, and lower) of the right and left lung is schematically represented for animals receiving 1×10^{7} - 10^{10}pfu/ml viral doses and necropsied on days 4 and 21. The degree of inflammation depicted for each field represents an averaging of the level of inflammation seen on multiple (2 to 4) sections from each lobe. As shown in Figure 12, CFTR recombinant adenoviral DNA was detectable only in the posterior segment of the upper right lobe and was confined to the area in which the Ad.CB-CFTR. was instilled. Comparison of signals detected in lung DNA samples to standards of known quantities of purified Ad.CB-CFTR DNA gave an estimated number of viral DNA molecules per cellular genome at day 4 post-infection as follows: $1x10^{10}$ pfu/ml - 10 viral DNA molecules per cellular genome; $1x10^{9}$pfu/ml-2 viral DNA molecules per cellular genome; $1x10^{8}$ pfu/ml - 0.2 viral DNA molecules per cellular genome; and $1x10^{7}$ pfu/ml - undetectable levels of viral DNA. No adenoviral DNA was detectable in lung samples at any of the doses in animals necropsied at day 21 with the exception of the $1x10^{9}$ pfu/ml dose in which trace amounts of DNA were visible on long exposures (less than 0.1 copies per cellular genome). In addition, no viral DNA was ever detected by Southern blot analysis in testes DNA harvested from animals necropsied at day 4 (all doses were analyzed).

To a varying degree and correlating with viral dose, the animals developed a lymphocytic perivascular infiltrate. At its mildest, small lymphocytic accumulations

were seen surrounding small to medium-sized vessels within lung parenchyma. Increased numbers of alveolar macrophages were also present. With increasing severity of the abnormality, the lymphocytic infiltrate extended beyond the immediate perivascular area and into the alveolar interstitial spaces. Intra-alveolar lymphocytes occasionally accompanied the increased number of macrophages. In lung regions having a higher intensity of pneumonitis, the alveolar tissue was densely infiltrated with mononuclear cells and at its worse, intra-alveolar edema could be seen. Figure 12 depicts the location and severity of the pneumonitis in the 8 animals on which detailed necropsies were performed. A grading system for the severity of inflammation was used to communicate the extent and location of inflammation: 1+, localized accumulations of lymphocytes in the immediate vicinity of small vessels with increase in intra-alveolar macrophages; 2+, inflammation extending out into neighboring alveolar septa; 3+, areas of confluent inflammation; and 4+, diffuse alveolar damage with intra-alveolar edema. Inflammation was absent or very mild in the lungs of all animals sacrificed on day 4 and in the lungs of animals receiving $1x10^7$ and 10^8 pfu/ml doses at day 21. Moderate to severe inflammation was seen in several areas of lung in the animal receiving $1x10^{10}$ pfu/ml and in one area of lung in the animal receiving $1x10^9$ pfu/ml dose. The inflammation was more likely present in regions of lung where virus was directly infused. However, it was also present outside these areas. This may represent spill over during instillation of virus since occasional Xgal positive cells were seen in frozen sections of lung tissue obtained from lung regions other than those directly infused. There seemed to be little if any difference in the degree of inflammation between the side receiving Ad.CMV-*lacZ* and the side receiving Ad.CB-CFTR. In scattered areas, eosinophils and rarely neutrophils could be seen. These cells were never the dominant type of inflammatory cell.

The majority of the inflammation was located within the distal lung parenchyma. There was no evidence of vessel wall necrosis although the lymphocytic infiltrate (1) seemed to arise around small vessels. The airways were relatively spared by the inflammation. Uncommonly, the well-demarcated bronchial associated lymphoid aggregates would spread to infiltrate bronchial wall. In almost all instances, the epithelium remained intact with preservation of its pseudocolumnar pattern with abundant ciliated cells.

In three animals, all captured in the wild, scattered aggregates of macrophages were found diffusely throughout the lung parenchyma. The

macrophages contained a dark greenish-black pigment that had within it refractile material suggestive of silicates. In one of the three animals, a small number of lymphocytes surrounded the macrophage aggregates. The source of the material is unknown.

Non-pulmonary organs. Grossly and microscopically, the remaining organs contained no abnormalities referable to gene treatment. In one of the wild-caught animals, parasitic cysts were seen in skeletal muscle, in bone marrow, and in two areas within the liver. (Tables I-X).

It will be appreciated that the non-human primate studies described herein are representative of current human studies involving the second generation adenovirus vectors of the present invention.

SPECIFIC EXAMPLE 4 - Human Protocol

The following is the protocol for human clinical trials for gene therapy using the vectors of the present invention containing an inserted CFTR gene. It will be appreciated that this protocol may also be used for gene therapy of genetic and epigenetic diseases other than cystic fibrosis.

Patient selection. Various criteria are used in evaluating cystic fibrosis patients for gene therapy using the adenovirus vectors of the present invention. The following criteria should be generally met by patients undergoing the clinical trials: (1) *Proven diagnosis of cystic fibrosis.* Proof will consist of documentation of both, sweat sodium or chloride greater than 60 mEq/l by the pilocarpine iontophoresis method or cystic fibrosis genotype and clinical manifestations of cystic fibrosis. (2) *Age.* Age greater than or equal to 18-years old. (3) *Gender.* Males or females may be used. Only patients who have no chance of procreating are entered into the study. Therefore, in the unlikely event that participation in the study induces mutations in the germline of the patient, these alterations will not be passed on to future generations. Males are eligible if they have documented azospermia. Over 95% of males with cystic fibrosis have congenital atrophy of the vas deferens and would thus fulfill this criterion. Females are eligible if they are documented to have had bilateral tubal ligations or a hysterectomy. (4) *Severity of disease.* To be eligible, a patient must be in adequate clinical condition to safely undergo the planned procedures, *i.e.* bronchoscopies. An acceptable reserve is defined as having a clinical condition such that the estimated 2-year survival is greater than 50%. Using the study of Kerem et al. (Kerem, E. et al., *N. Engl. J. Med.* 326:1187-1191 (1992)), patients are considered to have a greater than 50% chance of two

year survival if they fulfill all of the following: a) FEV_1 greater than or equal to 30% predicted; b) PaO_2 greater than 55 mmHg while breathing room air; and c) $PaCO_2$ less than 50 mmHg while breathing room air. Although the disease severity criteria have been selected to avoid entering patients with near terminal pulmonary disease, the intent nevertheless is to study those with at least moderate to severe lung disease and a substantially shortened predicted survival. To select only those with an estimated chance of 5-year survival of less than or equal to 50%, the patient must fulfill the following criterion (Shwachman, *Am. J. Dis. Child* 96:6-15 (1958)): d) Shwachman-Kulczycki clinical score less than or equal to 50.

Patients are excluded from clinical trials if they exhibit:

(1) *Risk of Complications.* Conditions which would place them at increased risk for complications from participating in the study. These conditions include: a) Pneumothorax within the last 12 months; b) Insulin-dependent diabetes; c) Asthma or allergic bronchopulmonary aspergillosis requiring glucocorticoid therapy within the last two months; d) Sputum culture growing a pathogen which does not have *in vitro* sensitivity to at least two types of antibiotics which could be administered to the patient; e) History of major hemoptysis: Coughing up greater that 250 ml of blood within a 24 hour period during the last year; and f) Any medical condition or laboratory abnormality which, according to the opinion of the investigators, would place the patient at increased risk for complications.

(2) *Evidence of Active Adenoviral Infection.* The patient is carefully evaluated for evidence of active adenoviral infection. History and physical examination is used to identify clinical evidence for adenoviral syndromes such as corya, pharyngitis, tonsillitis, bronchitis, pneumonia, conjunctivitis or diarrhea. A variety of specimens are evaluated for adenoviruses using culture techniques as well as immunofluorescent and enzyme-linked immunosorbent assays performed directly on the specimen. Evidence of active adenoviral infection at the time of therapy is a basis for exclusion. Another relevant question relates to previous exposure of the gene therapy recipient to Ad5 and similar serotypes based on adenovirus specific neutralizing antibodies. It is expected that virtually all adult patients will have been exposed to adenoviruses that confer humoral immunity to Ad5. This expectation is based on a large body of literature which indicate that the lower numbered adenoviruses Ad1, Ad2, Ad5, and Ad6 are endemic in most countries. In the United States, adenovirus-specific neutralizing antibodies are estimated to be present in 80% of individuals by the age of three. Sterner, *Acta Paediatr. Scand. Suppl.* 142-1

(1962); Hall, et al., *Am. J. Epichemiol.* 94:367 (1971); Foy, et al., "Viral Infections in Man," p. 5310 Ed. Evans AS Raven Press, NY (1976)). All patients enrolled in the protocol are evaluated for previous exposure to Ad5 and related types using a variety of serologic assays. It is expected that essentially every candidate patient will be seropositive. In fact, any patient who is not seropositive is excluded from the protocol. Existing humoral immunity to the virus is considered a safety feature which would prevent dissemination of the recombinant virus beyond the localized area of lung to which the recombinant virus is exposed.

(3) *Drug therapy.* Patients are excluded if they have been treated with systemic glucocorticoids within two months prior to initiation of the study.

(4) *Inability to comply with protocol.* Patients are excluded if, in the opinion of the investigators, the patient has characteristics which would make compliance with the protocol unlikely, *e.g.* drug abuse, alcoholism, psychiatric instability, inadequate motivation.

(5) *Participation in Other Studies.* Patients are excluded if they have participated in another investigational therapeutic study within the previous 90 days.

Patient evaluation. The following evaluations are performed at various times throughout the study: (1) *History and physical examination.* A history relevant to the manifestations of both cystic fibrosis and unrelated diseases is taken. A full review of systems, medication usage, and drug allergy history is obtained.

(2) *Clinical laboratory evaluations:* a) Blood: hemoglobin, hematocrit, white blood cell count, white blood cell differential count, platelet count, Westergren sedimentation rate, serum electrolytes (sodium, potassium, chloride, bicarbonate), BUN, creatinine, glucose, uric acid, total protein, albumin, calcium, phosphate, total bilirubin, conjugated bilirubin, AST, ALT, alkaline phosphatase, LDH; b) urine analysis: qualitative protein, blood, glucose, ketones, pH and microscopic examination.

(3) *Pulmonary function tests.* Testing should meet the standards set by the American Thoracic Society (1987a, 1987b): a) spirometry using the normal predicted values of Crapo et al. (1981); b) absolute lung volumes (total lung capacity, thoracic gas volume, residual volume); and c) diffusion capacity, single breath.

(4) *Arterial blood gases and pulse oximetry* - while breathing room air.

(5) *Electrocardiogram* (12-lead).

(6) *Postero-anterior and lateral chest X-ray.*

(7) *Thin-cut computerized tomography of the chest.*

(8) *Aerobic bacterial culture of sputum with antibiotic sensitivities.*

(9) *Shwachman-Kulczycki score calculation.*

(10) *Sperm count for males.* If a sperm count has not been done previously with the results documented, semen analysis is performed by the Department of Urology, University of Michigan Center.

(11) *Bronchoscopy.* Patients are allowed nothing by mouth for 6 hours prior to the procedure. They are premedicated with 0.2 mg glycopyrrolate and 50 mg meperidine intravenously 30 minutes before bronchoscopy. Electrocardiogram, pulse rate, and pulse oximetry is continuously monitored. Blood pressure is monitored every 5 minutes by an automated noninvasive system. Viscous lidocaine 2% (30 ml) is gargled and expectorated. Lidocaine 4% is sprayed onto the posterior pharynx and larynx by a hand held atomizer. The bronchoscope is introduced through the nose in patients without nasal obstruction or evidence of polyps. If the nasal approach cannot be used, the bronchoscope is introduced orally. In patients undergoing bronchoscopy by the nasal route, oxymetazoline hydrochloride 0.05% is applied topically to the mucosa of one nasal passage with a cotton swab. Lidocaine jelly 2% is instilled into the same nasal passage. Supplemental oxygen by cannula is administered at the mouth at 6 liters/minute. Midazolam is administered intravenously in 1 mg boluses over 15 seconds every 5 minutes until the patient is relaxed but still arousable by verbal stimuli. Additional midazolam is administered in 1 mg boluses up to every 15 minutes to maintain this level of sedation. A flexible fiberoptic bronchoscope is introduced transnassally. Lidocaine 2% is injected through the bronchoscope to anesthetize the larynx and airways as needed.

(12) *Bronchoalveolar lavage.* 50 ml aliquots of normal saline is injected through the bronchoscope that has been gently wedged into segmental bronchus. The lavagate is aspirated into a suction trap. The procedure is repeated until three aliquots have been administered and recovered.

(13) *Mucosal epithelial cell brushing.* A sleeved catheter with internal brush is introduced into the bronchus (Kelsen, *Am. J. Respir. Cell Mol. Bio.* 17:66-72 (1992)). The brush is rubbed against the epithelial mucosa and the adherent cells removed by agitating the brush in sterile medium.

(14) *Transbronchial biopsies.* A biopsy forceps is introduced into the bronchus and under fluoroscopic guidance six pieces of tissue are taken.

Clinical protocol. The following is the protocol for screening evaluation of the patient: Screening evaluation must be performed within four weeks of gene therapy. Written informed consent should be obtained prior to participation in the screening evaluation. Information obtained during screening is: history and physical exam; clinical laboratory blood tests; sperm count on males; pulmonary function tests; pulse oximetry and arterial blood gas; electrocardiography (12-lead); PA and lateral chest X-ray; thin-cut CT scan; sputum culture with antibiotic sensitivities; and Shwachman-Kulczycki Score calculation.

The following is the protocol for treatment of the patient prior to transfection: Beginning 2 weeks prior to transfection, the patient begins an intensified treatment protocol to reduce respiratory infection and maximize overall condition. For two weeks, the patient receives two anti-Pseudomonal antibiotics to which their cultured organism is sensitive. Twice a day postural drainage and percussion is performed. The patient continues on the remainder of their chronic treatment regimen. This phase is accomplished either as an inpatient or outpatient. During the subsequent studies, the patient continues on their previously prescribed medical program. This includes continuation of any oral antibiotics, pancreatic enzymes, theophylline, and vitamin supplements. Aerosolized bronchodilators and antibiotics are continued.

The following is the protocol for selection of the lung segment for transfection: The chest X-ray and thin cut CT scan is used to select an anatomical pulmonary segment that: a) has a degree of disease involvement average for that patient; and b) is in a location such that the patient can be positioned at bronchoscopy so that the segmental bronchus is gravitationally dependent.

The following is the transfection procedure: The patient is prepared for bronchoscopy as indicated above. The bronchoscope is introduced and advanced to the orifice of the segment chosen for the transfection. Secretions present within the segmental bronchus is aspirated through the bronchoscope. Transepithelial electrical potential difference is measured in the segment selected for transfection and in the segmental bronchus that is located in the same position in the opposite lung. A balloon catheter is introduced through the bronchoscope channel and advanced one centimeter into the orifice of the lung segment to be transfected. The balloon is inflated under direct vision until the orifice is minimally occluded. Fifty ml of virus at a concentration of $1x10^{10}$ pfu/ml in normal saline at 37°C is instilled through the balloon catheter. The catheter with balloon inflated is held in place for

30 minutes, after which time any remaining fluid is aspirated. The balloon is deflated and the catheter and bronchoscope removed.

A single dose of virus, $1x10^{10}$ pfu/ml in a total volume of 50 ml is used. This particular dose was selected based on experience with human CF xenografts. Englehardt et al., *Nature Genetics* 4:27-34 (1993). It has been found that increasing the concentration of virus above $1x10^{10}$ pfu/ml does not appreciably increase the efficiency of gene transfer. Lower doses are not used in this protocol because of the very real possibility that the efficiency of gene transfer would be insignificant.

The following is the protocol for post bronchoscopy monitoring: Vital signs including blood pressure, pulse, temperature, and respiratory rate are measured and recorded every five minutes for the first hour, every 15 minutes for the next two hours, every one hour for the next six hours, and every two hours for the next 15 hours, and every four hours for the rest of the week post-transfection. Continuous electrocardiographic and pulse oximetry are measured for the first 24 hours. The clinical laboratory blood tests that are listed above, pulse oximetry, and PA and lateral chest X-rays are performed daily for the first week, twice a week for the second week, and weekly thereafter for six weeks. Thin-cut CT scans are performed on the day prior to the follow-up bronchoscopy.

Following the administration of virus, the patients are kept in an isolation room with full respiratory precautions. The isolation room is a negative pressure room in which the air is filtered and delivered outside. Anyone entering the room wears a gown, mask, eye protection, and gloves. The patient is in isolation for at least 10 days after initiation of therapy. While in the hospital the patient has his or her sputum, nasal swab, urine and stool analyzed for replication competent adenoviruses of any serotype using standard adenovirus assays. These samples are also evaluated for CFTR recombinant virus using a PCR assay, known in the art. In the unlikely event that the patient continues to shed recombinant CFTR adenovirus in the airway, he or she is kept in the hospital for a longer period of time.

The following is a schedule for post-transfection bronchoscopies: Day 4 (transfection on day 0); Day 42; and Day 90.

The following samples and measurements are obtained during post-transfection bronchoscopies: a) transepithelial electrical potential difference at four sites within the transfected segment and within the segmental bronchus of its mirror image in the opposite lung: b) bronchoalveolar lavage of transfected segment and its mirror image in the opposite lung; c) six cytological brushings of alveolar surface

from the transfected segment; and d) six transbronchial biopsies from the transfected segment.

Evaluation of therapy. The patient is carefully monitored for toxicity, immunological response to CFTR protein or adenoviral proteins and efficiency and stability of gene transfer. The following protocol is followed: (1) *Toxicity.* Serial examinations to include PFT's, blood chemistry, hematology and cultures for adenovirus is performed. Bronchoalveolar lavage fluid obtained during each follow-up bronchoscopy is carefully analyzed for wild type and recombinant virus.

(2) *Immunological responses.* A major aspect of this protocol is to evaluate the serological response of the patient. The patient's serum and bronchoalveolar lavage fluid is evaluated for serological responses to wild type CFTR and adenoviral proteins. (3) *Efficiency and stability of gene transfer.* Bronchoscopy performed following gene transfer provides an opportunity to assess gene transfer and CFTR expression. Transepithelial electrical potential differences is performed at four sites within the transfected segment. Superficial airway epithelial cells are harvested by brushing and plated in culture. These cells are analyzed for CFTR protein and adenoviral protein expression using immunocytochemistry. Functional correction is assessed in cultured cells using the functional assay described. Transbronchial biopsy material, containing airway and airspace tissue, is analyzed for CFTR expression by immunocytochemistry and *in situ* hybridization.

SPECIFIC EXAMPLE 5 - Mutation and Selection Schemes

In addition to utilization of published temperature sensitive (*ts*) mutants, novel recombinant vectors are generated carrying *ts* mutations in the adenoviral genes E2, E4, L1, L2, L3, L4 and L5. Novel *ts* mutant adenoviral strains (potentially applying to all adenoviral serotypes including Ad2 through Ad41) are isolated as previously described. See Ensinger et al., *J. Virol.* 10:328-339 (1972). Stocks of wild type adenoviral DNA and/or virus are mutagenized by three different methods: 1) nitrous acid, 2) hydroxylamine, and 3) nitrosoguanidine. 293 cells are transfected or infected with mutated DNA or virus. Temperature sensitive mutant strains are isolated by a plaque enlargement technique in which mutagenized stocks are plaqued by agar overlay at 32°C. At day 14 post-infection plaques are stained in Neutral Red for 24 to 48 hr at 32°C (permissive temperature) and circumference of plaques outlined. Plates are then shifted to 39.5°C (non-permissive temperature) for 48 hr and only plaques which do not enlarge are picked for screening for their ability to cause complete temperature-sensitive CPE on HeLa cells. The *ts* mutants

thus derived are classified by functional complementation of viral strains carrying known mutations. Once potentially useful *ts* mutants have been identified, they are cloned by homologous recombination into recombinant vectors (as described above) to give new *ts* recombinant adenoviral stocks. In addition, recombinant adenovirus containing multiple *ts* mutations may be generated.

Those skilled in the art can now appreciate from the foregoing description that the broad teachings of the present invention can be implemented in a variety of forms. Therefore, while this invention has been described in connection with particular examples thereof, the true scope of the invention should not be so limited since other modifications will become apparent to the skilled practitioner upon a study of the specification and following claims.

All publications and applications cited herein are incorporated by reference.

Table I - Animal Identification and Viral Doses

Study #	Animal ID	Dose pfu/ml	Species	Sex	Wt lbs	Date of Birth (Age)
I	CFB1	1.0E+10	*P. anubis*	M	71.5	Unknown (approx. 12 yr)
II	CFB2	1.0E+10	*P. cynocephalus/anubis*	M	71.5	Unknown (approx. 12 yr)
III	CFB3	1.0E+07	*P. papio*	M	23	10/01/88
	CFB5	1.0E+07	*P. papio*	M	26.5	09/12/88
	CFB7	1.0E+07	*P. papio*	M	25	12/31/87
	CFB10	1.0E+08	*P. papio*	M	13.5	09/11/90
	CFB14	1.0E+08	*P. papio*	M	19.2	Unknown (2-5 yr)
	CFB16	1.0E+08	*P. papio*	M	20.3	Unknown (2-5 yr)
	CFB11	1.0E+09	*P. papio*	M	14.7	10/25/90
	CFB13	1.0E+09	*P. papio*	M	19.4	Unknown (2-5 yr)
	CFB15	1.0E+09	*P. papio*	M	20.5	Unknown (2-5 yr)
	CFB4	1.0E+10	*P. papio*	M	17	Unknown (2-5 yr)
	CFB6	1.0E+10	*P. papio*	M	30	Unknown (2-5 yr)
	CFB8	1.0E+10	*P. papio*	M	23	Unknown (2-5 yr)

Table IIa - Hematology for Baboon Toxicity Studies

	HGB	HCT	PLATELETS	WBC	SEGS
NORMAL ADULT BABOON	12.1-15.3	38-48	205-451	5.9-20.8	22-85
NORMAL JUVENILE BABOON	8.7-13.9	31-43	225-544	3.3-19.0	23-78
	STABS	LYMPHS	MONO	EOSIN	BASO
NORMAL ADULT BABOON	0-4	12.0-75	0-4	0-5	0-1
NORMAL JUVENILE BABOON	0-6	14-76	0-3	0-8	0-1
	PTT	PT	INR		
NORMAL ADULT BABOON	N/A	N/A	N/A		
NORMAL JUVENILE BABOON	N/A	N/A	N/A		

Table IIb - Hematology for Baboon Toxicity Studies - Study I

VIRUS	NECROPSY	ANIMAL	DATE	DAY	HGB	HCT	PLATELETS	WBC
1.0E+10	4 DAY	CFB1	ND					
VIRUS	NECROPSY	ANIMAL	DATE	DAY	SEGS	STABS	LYMPHS	MONO
1.0E+10	4 DAY	CFB1	ND					
VIRUS	NECROPSY	ANIMAL	DATE	DAY	EOSIN	BASO	PTT	PT
1.0E+10	4 DAY	CFB1	ND					
VIRUS	NECROPSY	ANIMAL	DATE	DAY	INR			
1.0E+10	4 DAY	CFB1	ND					

Table IIc -Hematology for Baboon Toxicity Studies - Study II

VIRUS	NECROPSY	ANIMAL	DATE	DAY	HGB	HCT	PLATELETS	WBC
1.0E+10	LONG-TERM	CFB2	11/06/92	-3	14.0	43.2	273	8.3
			11/09/92	0	13.9	40.9	296	7.1
			11/12/92	3	13.8	40.6	256	5.7
			11/23/92	14	14.2	41.5	428	9.8
			11/30/92	21	13.8	42.4	375	10.1
			01/21/93	73	14.1	42.2	321	12.0
			01/25/93	77	13.8	40.8	338	8.7
			02/19/93	102	14.2	42.4	300	7.3
			03/05/93	116	13.8	41.1	287	5.5
			04/10/93	152	10.0	41.6	333	10.0

VIRUS	NECROPSY	ANIMAL	DATE	DAY	SEGS	STABS	LYMPHS	MONO
1.0E+10	LONG-TERM	CFB2	11/06/92	-3	62	2	33	2
			11/09/92	0	74	-	19	5
			11/12/92	3	63	0	25	11
			11/23/92	14	66	-	31	1
			11/30/92	21	60	-	31	3
			01/21/93	73	80	-	18	1
			01/25/93	77	71	-	24	4
			02/19/93	102	61	-	33	5
			03/05/93	116	58	-	36	5
			04/10/93	152	77	-	20	3

Table IIc -Hematology for Baboon Toxicity Studies - Study II (cont.)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	EOSIN	BASO	PTT	PT
1.0E+10	LONG-TERM	CFB2	11/06/92	-3	1	0	N D	N D
			11/09/92	0	1	2	N D	N D
			11/12/92	3	1	0	27.8	12.0
			11/23/92	14	1	1	29.1	11.8
			11/30/92	21	2	4	27.0	12.5
			01/21/93	73	0	0	N D	N D
			01/25/93	77	1	0	N D	N D
			02/19/93	102	1	0	N D	N D
			03/05/93	116	1	0	25.0	12.2
			04/10/93	152	1	0		

VIRUS	NECROPSY	ANIMAL	DATE	DAY	INR
1.0E+10	LONG-TERM	CFB2	11/06/92	-3	N D
			11/09/92	0	N D
			11/12/92	3	0.9
			11/23/92	14	0.9
			11/30/92	21	1.0
			01/21/93	73	N D
			01/25/93	77	N D
			02/19/93	102	N D
			03/05/93	116	0.9
			04/10/93	152	

Table IId - Hematology for Baboon Toxicity Studies - Study III

VIRUS	NECROPSY	ANIMAL	DATE	DAY	HGB	HCT	PLATELETS	WBC
1.0E+07	4 DAY	CFB3	01/18/93	0	13.3	38.9	298	6.7
			01/22/93	4	12.9	37.8	313	5.6
	21 DAY	CFB5	01/18/93	0	13.5	40.2	404	11.6
			01/22/93	4	12.5	36.0	444	8.1
			02/02/93	15	12.8	38.2	421	12.1
			02/08/93	21	12.5	37.8	442	6.6
	LONG-TERM	CFB7	01/18/93	0	13.6	39.5	256	10.3
			01/22/93	4	12.7	38.1	238	6.7
			02/02/93	15	13.2	39.7	322	8.0
			02/08/93	21	12.7	38.6	319	7.2
			03/10/93	51	13.8	41.2	272	6.3
			04/10/93	82	13.9	41.2	324	5.9

VIRUS	NECROPSY	ANIMAL	DATE	DAY	SEGS	STABS	LYMPHS	MONO
1.0E+07	4 DAY	CFB3	01/18/93	0	46	-	45	4
			01/22/93	4	53	1	41	2
	21 DAY	CFB5	01/18/93	0	74	0	26	0
			01/22/93	4	58	-	38	2
			02/02/93	15	79	-	19	1
			02/08/93	21	35	0	60	2
	LONG-TERM	CFB7	01/18/93	0	67	-	28	4
			01/22/93	4	52	-	40	6
			02/02/93	15	50	3	38	8
			02/08/93	21	55	0	43	2
			03/10/93	51	49	1	45	4
			04/10/93	82	28		53	16

Table IId - Hematology for Baboon Toxicity Studies - Study III (cont.)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	EOSIN	BASO	PTT	PT
1.0E+07	4 DAY	CFB3	01/18/93	0	3	3	N D	N D
			01/22/93	4	1	0	23.8	13.2
	21 DAY	CFB5	01/18/93	0	0	0	23.0	13.4
			01/22/93	4	2	0	23.8	12.8
			02/02/93	15	1	0	25.1	12.9
			02/08/93	21	3	0	22.9	12.4
	LONG-TERM	CFB7	01/18/93	0	1	0	24.7	13.2
			01/22/93	4	2	0	23.1	12.7
			02/02/93	15	1	0	27.0	13.0
			02/08/93	21	0	0	24.6	12.9
			03/10/93	51	1	0	ND	ND
			04/10/93	82	3	0		

VIRUS	NECROPSY	ANIMAL	DATE	DAY	INR
1.0E+07	4 DAY	CFB3	01/18/93	0	N D
			01/22/93	4	1.1
	21 DAY	CFB5	01/18/93	0	1.1
			01/22/93	4	1.0
			02/02/93	15	1.1
			02/08/93	21	1.0
	LONG-TERM	CFB7	01/18/93	0	1.1
			01/22/93	4	1.0
			02/02/93	15	1.1
			02/08/93	21	1.1
			03/10/93	51	ND
			04/10/93	82	

Table IIe - Hematology for Baboon Toxicity Studies - Study III

VIRUS	NECROPSY	ANIMAL	DATE	DAY	HGB	HCT	PLATELETS	WBC	SEGS
1.0E+08	4 DAY	CFB10	02/03/93	-13	13.0	39.5	244	14.3	39
			02/10/93	-6	12.4	37.3	295	10.1	44
			02/16/93	0	12.5	37.7	236	6.9	27
			02/20/93	4	12.5	37.4	280	5.6	22
	21 DAY	CFB14	01/29/93	-18	12.0	35.5	338	5.8	53
			02/16/93	0	12.3	36.3	CLUMPED	9.5	63
			02/20/93	4	12.1	35.9	251	6.8	52
			03/02/93	14	12.5	38.0	CLUMPED	13.7	63
			03/09/93	21	12.6	37.6	CLUMPED	9.9	68
	LONG-TERM	CFB16	01/29/93	-18	12.2	36.7	CLUMPED	7.6	42
			02/16/93	0	12.7	38.9	CLUMPED	10.0	52
			02/20/93	4	11.7	35.1	268	5.6	19
			03/02/93	14	11.0	33.6	240	6.8	46
			03/09/93	21	11.1	33.1	211	8.0	45
			04/10/93	53	11.7	35.0	311	9.5	42

VIRUS	NECROPSY	ANIMAL	DATE	DAY	STABS	LYMPHS	MONO	EOSIN	BASO
1.0E+08	4 DAY	CFB10	02/03/93	-13	0	55	4	2	0
			02/10/93	-6	1	49	3	3	0
			02/16/93	0	0	68	2	3	0
			02/20/93	4	0	69	4	5	0
	21 DAY	CFB14	01/29/93	-18	-	42	4	1	0
			02/16/93	0	0	36	1	0	0
			02/20/93	4	0	45	2	0	1
			03/02/93	14	0	33	3	1	0
			03/09/93	21	1	29	1	0	0

Table IIe - Hematology for Baboon Toxicity Studies - Study III (cont.)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	STABS	LYMPHS	MONO	EOSIN	BASO
	LONG-TERM	CFB16	01/29/93	-18	0	47	3	3	0
			02/16/93	0	0	47	1	0	0
			02/20/93	4	0	74	4	3	0
			03/02/93	14	4	45	5	0	0
			03/09/93	21	0	47	7	1	0
			04/10/93	53	54	4	0	0	0

VIRUS	NECROPSY	ANIMAL	DATE	DAY	PTT	PT	INR		
1.0E+08	4 DAY	CFB10	02/03/93	-13	ND	ND	ND		
			02/10/93	-6	ND	ND	ND		
			02/16/93	0	24.7	13.0	1.1		
			02/20/93	4	24.0	13.1	1.0		
	21 DAY	CFB14	01/29/93	-18	ND	ND	ND		
			02/16/93	0	25.0	12.5	1.0		
			02/20/93	4	24.8	12.1	0.9		
			03/02/93	14	24.5	12.2	1.0		
			03/09/93	21	25.1	12.1	0.9		
	LONG-TERM	CFB16	01/29/93	-18	ND	ND	ND		
			02/16/93	0	22.0	12.1	0.9		
			02/20/93	4	22.6	12.2	1.0		
			03/02/93	14	22.3	12.5	1.0		
			03/09/93	21	22.4	11.9	0.9		
			04/10/93	53					

Table IIf - Hematology for Baboon Toxicity Studies - Study III

VIRUS	NECROPSY	ANIMAL	DATE	DAY	HGB	HCT	PLATELETS	WBC	SEGS
1.0E+09	4 DAY	CFB11	02/03/93	-13	12.6	38.5	337	6.1	69
			02/16/93	0	12.9	38.5	313	5.5	54
			02/20/93	4	12.4	37.6	315	4.9	58
	21 DAY	CFB13	01/29/93	-18	12.3	36.6	447	5.5	39
			02/16/93	0	12.8	38.3	269	6.1	40
			02/20/93	4	12.8	38.3	403	5.9	61
			03/02/93	14	13.1	39.5	385	6.7	52
			03/09/93	21	12.9	38.4	305	6.1	46
	LONG-TERM	CFB15	02/03/93	-13	13.0	39.2	332	4.4	51
			02/16/93	0	12.9	38.8	322	8.7	78
			02/20/93	4	12.2	37.7	352	5.5	53
			03/02/93	14	12.1	37.2	334	9.9	73
			03/09/93	21	12.6	38.2	224	7.3	56
			04/10/93	53	13.2	39.5	259	5.7	48

VIRUS	NECROPSY	ANIMAL	DATE	DAY	STABS	LYMPHS	MONO	EOSIN	BASO
1.0E+09	4 DAY	CFB11	02/03/93	-13	-	28	2	0	1
			02/16/93	0	-	44	1	1	0
			02/20/93	4	-	38	3	1	1
	21 DAY	CFB13	01/29/93	-18	0	51	7	3	0
			02/16/93	0	0	50	1	0	0
			02/20/93	4	0	34	5	0	0
			03/02/93	14	0	44	2	1	1
			03/09/93	21	0	48	4	2	0

Table IIf - Hematology for Baboon Toxicity Studies - Study III (cont.)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	STABS	LYMPHS	MONO	EOSIN	BASO
1.0E+09	LONG-TERM	CFB15	02/03/93	-13	-	41	5	2	1
			02/16/93	0	0	17	4	1	0
			02/20/93	4	0	38	7	1	1
			03/02/93	14	-	20	6	1	0
			03/09/93	21	2	34	3	4	1
			04/10/93	53		44	4	3	1
VIRUS	NECROPSY	ANIMAL	DATE	DAY	PTT	PT	INR		
1.0E+09	4 DAY	CFB11	02/03/93	-13	ND	ND	ND		
			02/16/93	0	25.4	12.6	1.0		
			02/20/93	4	24.2	12.3	1.0		
	21 DAY	CFB13	01/29/93	-18	ND	ND	ND		
			02/16/93	0	N A	13.9	1.2		
			02/20/93	4	25.6	12.3	1.0		
			03/02/93	14	25.3	12.4	1.0		
			03/09/93	21	23.8	12.0	0.9		
	LONG-TERM	CFB15	02/03/93	-13	ND	ND	ND		
			02/16/93	0	23.1	13.1	1.1		
			02/20/93	4	24.1	13.1	1.1		
			03/02/93	14	24.2	13.7	1.2		
			03/09/93	21	22.8	12.6	1.0		
			04/10/93	53					

Table IIg - Hematology for Baboon Toxicity Studies - Study III

VIRUS	NECROPSY	ANIMAL	DATE	DAY	HGB	HCT	PLATELETS	WBC	SEGS
1.0E+10	4 DAY	CFB4	01/18/93	0	13.4	39.3	342	9.7	29
			01/22/93	4	12.5	36.8	338	8.9	25
	21 DAY	CFB6	01/18/93	0	12.8	37.7	364	8.8	67
			01/22/93	4	12.8	38.1	341	8.2	60
			02/02/93	15	12.0	36.1	343	8.9	63
			02/08/93	21	12.3	37.6	425	9.6	74
	LONG-TERM	CFB8	01/18/93	0	12.9	39.4	372	8.2	48
			01/22/93	4	12.6	37.8	347	6.3	25
			02/02/93	15	13.2	40.3	387	10.6	63
			02/08/93	21	12.9	39.7	462	9.8	50
			03/10/93	51	12.7	38.6	381	10.3	64
			04/10/93	82	13.2	39.9	429	9.1	45

VIRUS	NECROPSY	ANIMAL	DATE	DAY	STABS	LYMPHS	MONO	EOSIN	BASO
1.0E+10	4 DAY	CFB4	01/18/93	0	0	67	3	1	0
			01/22/93	4	0	71	4	0	0
	21 DAY	CFB6	01/18/93	0	-	28	4	1	0
			01/22/93	4	-	36	2	1	1
			02/02/93	15	6	25	6	0	0
			02/08/93	21	-	24	2	1	0
	LONG-TERM	CFB8	01/18/93	0	0	48	1	2	1
			01/22/93	4	0	68	3	3	0
			02/02/93	15	-	32	2	2	1
			02/08/93	21	0	47	2	1	0
			03/10/93	51	-	30	2	1	3
			04/10/93	82		42	11	2	

Table IIg - Hematology for Baboon Toxicity Studies - Study III (cont.)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	PTT	PT	INR		
1.0E+10	4 DAY	CFB4	01/18/93	0	26.6	12.9	1.1		
			01/22/93	4	26.0	12.4	1.0		
	21 DAY	CFB6	01/18/93	0	24.8	13.2	1.1		
			01/22/93	4	22.3	12.8	1.0		
			02/02/93	15	24.7	13.0	1.1		
			02/08/93	21	23.4	12.8	1.0		
	LONG-TERM	CFB8	01/18/93	0	26.8	13.3	1.1		
			01/22/93	4	24.4	12.4	1.0		
			02/02/93	15	24.1	12.8	1.1		
			02/08/93	21	24.9	12.7	1.0		
			03/10/93	51	ND	ND	ND		
			04/10/93	82					

Table IIIa - Blood Chemistries for Toxicity Studies

	SODIUM	POTASSIUM	CHLORIDE	HCO3
NORMAL ADULT BABOON	147-153	3.6-4.9	100-108	N/A
NORMAL JUVENILE BABOON	143-158	3.2-4.3	104-118	N/A
	BUN	CREATININE	GLUCOSE	CALCIUM
NORMAL ADULT BABOON	16-22	1.3-1.9	57-120	7.1-10.1
NORMAL JUVENILE BABOON	9.0-25	0.8-1.4	50-129	8.0-9.6
	PHOSPHOROUS	T. PROTEIN	ALBUMIN	GLOBULIN
NORMAL ADULT BABOON	2.2-5.3	5.7-7.7	2.8-3.9	2.8-4.1
NORMAL JUVENILE BABOON	4.7-7.7	5.8-7.8	2.9-4.2	2.4-4.4
	SGOT	SGPT	LDH	ALK.PHOS.
NORMAL ADULT BABOON	19-34	20-62	123-327	82-209
NORMAL JUVENILE BABOON	16-39	12.0-81	99-488	154-1105
	T.BILIRUBIN	CHOLESTEROL		
NORMAL ADULT BABOON	0.4	68-111		
NORMAL JUVENILE BABOON	0.3-0.7	68-232		

Table IIIb - Blood Chemistries for Toxicity Studies - Study I (Virus = 1.0E+10)

NECROPSY	ANIMAL	DATE	DAY	SODIUM	POTASSIUM	CHLORIDE	HCO3
4 DAY	CFB1	10/20/92	-2	145	3.6	102	25
NECROPSY	ANIMAL	DATE	DAY	BUN	CREATININE	GLUCOSE	CALCIUM
4 DAY	CFB1	10/20/92	-2				
NECROPSY	ANIMAL	DATE	DAY	PHOSPHORUS	T.PROTEIN	ALBUMIN	GLOBULIN
4 DAY	CFB1	10/20/92	-2				
NECROPSY	ANIMAL	DATE	DAY	SGOT	SGPT	LDH	ALK.PHOS.
4 DAY	CFB1	10/20/92	-2				
NECROPSY	ANIMAL	DATE	DAY	T.BILIRUBIN	CHOLESTEROL		
4 DAY	CFB1	10/20/92	-2				

Table IIIc- Blood Chemistries for Toxicity Studies

NECROPSY	ANIMAL	DATE	DAY	SODIUM	POTASSIUM	CHLORIDE	HCO3
LONG-TERM	CFB2	11/06/92	-3	142	3.1	110	25
		11/09/92	0	147	4.3	107	28
		11/12/92	3	142	4.5	104	31
		11/23/92	14	147	4.6	107	27
		11/30/92	21	150	4.2	106	31
		01/21/93	73	147	3.5	102	29
		01/25/93	77	144	4.0	102	29
		02/19/93	102	144	3.9	101	29
		03/05/93	116	147	3.9	104	28
		04/10/93	152	146	3.8	105	29
NECROPSY	ANIMAL	DATE	DAY	BUN	CREATININE	GLUCOSE	CALCIUM
LONG-TERM	CFB2	11/06/92	-3	13	1.3	71	9.8
		11/09/92	0	19	1.3	58	9.3
		11/12/92	3	16	1.2	83	9.0
		11/23/92	14	19	1.1	75	9.3
		11/30/92	21	23	1.1	66	9.5
		01/21/93	73	24	1.1	34	9.4
		01/25/93	77	23	1.2	62	9.2
		02/19/93	102	22	1.1	59	9.2
		03/05/93	116	26	1.1	71	9.5
		04/10/93	152	27	1.2	53	9.2
NECROPSY	ANIMAL	DATE	DAY	PHOSPHORUS	T.PROTEIN	ALBUMIN	GLOBULIN
LONG-TERM	CFB2	11/06/92	-3	4.3	7.2	4.4	-4.4
		11/09/92	0	6.3	6.5	3.8	-3.8
		11/12/92	3	5.1	6.1	3.1	-3.1
		11/23/92	14	5.8	6.9	3.5	-3.5
		11/30/92	21	4.7	6.5	3.5	-3.5

Table IIIc- Blood Chemistries for Toxicity Studies (cont.)

		01/21/93	73	4.0	7.1	4.1	-4.1
		01/25/93	77	4.1	6.6	3.6	-3.6
		02/19/93	102	4.5	6.7	3.8	2.9
		03/05/93	116	4.3	6.2	3.6	2.6
		04/10/93	152	4.1	6.7	3.8	2.9
NECROPSY	**ANIMAL**	**DATE**	**DAY**	**SGOT**	**SGPT**	**LDH**	**ALK.PHOS.**
LONG-TERM	CFB2	11/06/92	-3	122	121	237	280
		11/09/92	0	56	85	501	130
		11/12/92	3	62	66	655	133
		11/23/92	14	50	110	457	490
		11/30/92	21	44	96	472	338
		01/21/93	73	47	57	762	222
		01/25/93	77	34	74	235	250
		02/19/93	102	63	100	334	200
		03/05/93	116	20	57	134	170
		04/10/93	152	46	95	203	214
NECROPSY	**ANIMAL**	**DATE**	**DAY**	**T.BILIRUBIN**	**CHOLESTEROL**		
LONG-TERM	CFB2	11/06/92	-3	0.2	70		
		11/09/92	0	0.2	83		
		11/12/92	3	0.3	68		
		11/23/92	14	0.2	92		
		11/30/92	21	0.2	81		
		01/21/93	73	0.2	82		
		01/25/93	77	0.4	84		
		02/19/93	102	0.5	75		
		03/05/93	116	0.2	70		
		04/10/93	152	0.5	76		

Table IIId - Blood Chemistries for Toxicity Studies - Study III (Virus = 1.0E+07)

NECROPSY	ANIMAL	DATE	DAY	SODIUM	POTASSIUM	CHLORIDE	HCO3
4 DAY	CFB3	01/18/93	0	149	3.5	100	31
		01/22/93	4	148	4.4	102	34
21 DAY	CFB5	01/18/93	0	159	3.8	110	34
		01/22/93	4	149	3.6	102	32
		02/02/93	15	148	3.5	102	32
		02/08/93	21	149	3.1	101	31
LONG-TERM	CFB7	01/18/93	0	146	3.9	103	28
		01/22/93	4	148	3.8	102	36
		02/02/93	15	143	4.0	99	28
		02/08/93	21	148	3.6	100	29
		03/10/93	51	146	3.6	101	32
		04/10/93	82	146	3.5	102	28

NECROPSY	ANIMAL	DATE	DAY	BUN	CREATININE	GLUCOSE	CALCIUM
4 DAY	CFB3	01/18/93	0	18	0.6	68	10.2
		01/22/93	4	16	0.7	52	10.3
21 DAY	CFB5	01/18/93	0	27	1.0	85	10.1
		01/22/93	4	17	0.8	84	9.9
		02/02/93	15	16	0.7	65	9.6
		02/08/93	21	18	0.7	86	9.7
LONG-TERM	CFB7	01/18/93	0	24	0.8	80	9.5
		01/22/93	4	21	0.7	80	9.5
		02/02/93	15	20	0.8	61	9.1
		02/08/93	21	18	0.7	60	9.3
		03/10/93	51	18	0.7	67	9.4
		04/10/93	82	21	0.7	70	8.9

Table IIId - Blood Chemistries for Toxicity Studies - Study III (Virus = 1.0E+07) (cont.)

NECROPSY	ANIMAL	DATE	DAY	PHOSPHORUS	T.PROTEIN	ALBUMIN	GLOBULIN
4 DAY	CFB3	01/18/93	0	6.0	7.5	4	3.5
		01/22/93	4	5.9	6.8	3.9	2.9
21 DAY	CFB5	01/18/93	0	6.0	7.1	4	3.1
		01/22/93	4	4.3	7.1	4.2	2.9
		02/02/93	15	5.8	6.8	3.9	2.9
		02/08/93	21	5.2	6.6	3.7	2.9
LONG-TERM	CFB7	01/18/93	0	6.1	6.5	3.7	2.8
		01/22/93	4	5.7	6.5	3.6	2.9
		02/02/93	15	5.1	7	3.8	3.2
		02/08/93	21	6.2	6.7	3.7	3.0
		03/10/93	51	6.1	6.3	3.6	2.7
		04/10/93	82	5.7	6.5	3.6	2.9

NECROPSY	ANIMAL	DATE	DAY	SGOT	SGPT	LDH	ALK.PHOS.
4 DAY	CFB3	01/18/93	0	46	28	406	706
		01/22/93	4	45	29	438	676
21 DAY	CFB5	01/18/93	0	17	14	217	772
		01/22/93	4	25	20	320	702
		02/02/93	15	34	23	332	727
		02/08/93	21	36	20	455	632
LONG-TERM	CFB7	01/18/93	0	24	19	232	1127
		01/22/93	4	28	20	299	862
		02/02/93	15	32	18	296	991
		02/08/93	21	26	20	272	872
		03/10/93	51	29	19	255	967
		04/10/93	82	19	9	229	824

Table IIId - Blood Chemistries for Toxicity Studies - Study III (Virus = 1.0E+07) (cont.)

NECROPSY	ANIMAL	DATE	DAY	T.BILIRUBIN	CHOLESTEROL		
4 DAY	CFB3	01/18/93	0	0.2	100		
		01/22/93	4	0.2	83		
21 DAY	CFB5	01/18/93	0	0.2	95		
		01/22/93	4	0.2	86		
		02/02/93	15	0.2	84		
		02/08/93	21	0.2	79		
LONG-TERM	CFB7	01/18/93	0	0.3	80		
		01/22/93	4	0.2	86		
		02/02/93	15	0.2	86		
		02/08/93	21	0.2	81		
		03/10/93	51	0.2	84		
		04/10/93	82	0.2	84		

Table IIIe - Blood Chemistries for Toxicity Studies

NECROPSY	ANIMAL	DATE	DAY	SODIUM	POTASSIUM	CHLORIDE	HCO3
4 DAY	CFB10	02/03/93	-13	147	3.6	101	19
		02/10/93	-6	146	3.4	102	23
		02/16/93	0	147	3.4	102	29
		02/20/93	4	146	3.3	101	27
21 DAY	CFB14	01/29/93	-18	146	4.3	104	25
		02/16/93	0	146	3.1	101	28
		02/20/93	4	145	3.6	100	28
		03/02/93	14	145	3.6	101	24
		03/09/93	21	144	3.5	100	27
LONG-TERM	CFB16	01/29/93	-18	146	3.7	104	16
		02/16/93	0	147	4.0	102	24
		02/20/93	4	144	3.9	99	25
		03/02/93	14	144	4.0	101	26
		03/09/93	21	146	4.0	102	24
		04/10/93	53	144	3.6	104	25

NECROPSY	ANIMAL	DATE	DAY	BUN	CREATININE	GLUCOSE	CALCIUM
4 DAY	CFB10	02/03/93	-13	14	0.6	110	9.1
		02/10/93	-6	23	0.6	87	9.3
		02/16/93	0	19	0.6	10	9.6
		02/20/93	4	15	0.7	77	9.1
21 DAY	CFB14	01/29/93	-18	8	0.5	92	10.1
		02/16/93	0	11	0.4	69	10.1
		02/20/93	4	11	0.5	90	10.1
		03/02/93	14	12	0.5	90	10.0
		03/09/93	21	12	0.6	82	10.0

Table IIIe - Blood Chemistries for Toxicity Studies (cont.)

LONG-TERM	CFB16	01/29/93	-18	9	0.7	98	9.6
		02/16/93	0	10	0.6	72	10.4
		02/20/93	4	13	0.5	76	10.1
		03/02/93	14	15	0.5	69	9.8
		03/09/93	21	14	0.5	65	10.3
		04/10/93	53	15	0.6	74	9.9
NECROPSY	**ANIMAL**	**DATE**	**DAY**	**PHOSPHORUS**	**T.PROTEIN**	**ALBUMIN**	**GLOBULIN**
4 DAY	CFB10	02/03/93	-13	4.7	7.2	3.6	3.6
		02/10/93	-6	4.4	6.9	3.5	3.4
		02/16/93	0	3.9	6.7	3.5	3.2
		02/20/93	4	5.2	6.7	3.4	3.3
21 DAY	CFB14	01/29/93	-18	6.4	7.5	3.9	3.6
		02/16/93	0	7.1	7	3.7	3.3
		02/20/93	4	6.7	7	3.8	3.2
		03/02/93	14	7.0	6.9	3.8	3.1
		03/09/93	21	6.7	6.8	3.8	3.0
LONG-TERM	CFB16	01/29/93	-18	5.8	7.4	4.4	3.0
		02/16/93	0	7.3	7.8	4.6	3.2
		02/20/93	4	6.1	7.4	4.2	3.2
		03/02/93	14	6.1	7.2	4.0	3.2
		03/09/93	21	5.6	7.2	4.1	3.1
		04/10/93	53	5.3	6.9	4.0	2.9
NECROPSY	**ANIMAL**	**DATE**	**DAY**	**SGOT**	**SGPT**	**LDH**	**ALK.PHOS.**
4 DAY	CFB10	02/03/93	-13	33	25	328	633
		02/10/93	-6	20	21	227	620
		02/16/93	0	36	29	326	592

Table IIIe - Blood Chemistries for Toxicity Studies (cont.)

		02/20/93	4	20	25	234	639
21 DAY	CFB14	01/29/93	-18	28	32	285	771
		02/16/93	0	28	74	256	663
		02/20/93	4	16	41	173	703
		03/02/93	14	15	43	179	874
		03/09/93	21	23	41	180	902
LONG-TERM	CFB16	01/29/93	-18	22	29	208	630
		02/16/93	0	17	34	230	581
		02/20/93	4	11	28	188	493
		03/02/93	14	16	32	191	600
		03/09/93	21	18	35	180	594
		04/10/93	53	17	25	168	673

NECROPSY	ANIMAL	DATE	DAY	T.BILIRUBIN	CHOLESTEROL		
4 DAY	CFB10	02/03/93	-13	0.3	102		
		02/10/93	-6	0.1	105		
		02/16/93	0	0.1	102		
		02/20/93	4	0.2	107		
21 DAY	CFB14	01/29/93	-18	0.5	156		
		02/16/93	0	0.3	140		
		02/20/93	4	0.2	140		
		03/02/93	14	0.2	128		
		03/09/93	21	0.2	124		
LONG-TERM	CFB16	01/29/93	-18	0.5	202		
		02/16/93	0	0.2	177		
		02/20/93	4	0.2	137		
		03/02/93	14	0.2	139		
		03/09/93	21	0.2	136		
		04/10/93	53	0.3	131		

Table IIIf - Blood Chemistries for Toxicity Studies - Study III (Virus = 1.0E+09)

NECROPSY	ANIMAL	DATE	DAY	SODIUM	POTASSIUM	CHLORIDE	HCO3
4 DAY	CFB11	02/03/93	-13	150	3.8	103	17
		02/16/93	0	147	4.1	101	15
		02/20/93	4	148	4.0	101	19
21 DAY	CFB13	01/29/93	-18	147	4.0	104	27
		02/16/93	0	148	3.5	103	28
		02/20/93	4	143	3.8	99	27
		03/02/93	14	145	4.1	100	27
		03/09/93	21	146	3.6	101	29
LONG-TERM	CFB15	02/03/93	-13	146	4.2	101	22
		02/16/93	0	147	4.3	10.3	17
		02/20/93	4	147	4.2	102	19
		03/02/93	14	147	4.0	102	25
		03/09/93	21	147	4.3	103	18
		04/10/93	53	145	3.7	103	21

NECROPSY	ANIMAL	DATE	DAY	BUN	CREATININE	GLUCOSE	CALCIUM
4 DAY	CFB11	02/03/93	-13	18	0.9	90	10.1
		02/16/93	0	21	0.9	58	10.3
		02/20/93	4	14	0.8	80	10.0
21 DAY	CFB13	01/29/93	-18	13	0.8	88	9.7
		02/16/93	0	15	0.6	62	9.7
		02/20/93	4	16	0.7	66	9.7
		03/02/93	14	17	0.6	63	9.9
		03/09/93	21	17	0.6	56	10.1
LONG-TERM	CFB15	02/03/93	-13	22	0.9	100	10.2
		02/16/93	0	18	0.7	83	10.0
		02/20/93	4	18	0.7	91	9.5
		03/02/93	14	18	0.7	69	9.6
		03/09/93	21	22	0.7	73	10.0
		04/10/93	53	22	0.7	79	9.8

Table IIIf - Blood Chemistries for Toxicity Studies - Study III (Virus = 1.0E+09) (cont.)

NECROPSY	ANIMAL	DATE	DAY	PHOSPHORUS	T.PROTEIN	ALBUMIN	GLOBULIN
4 DAY	CFB11	02/03/93	-13	6.8	7.1	4.1	3.0
		02/16/93	0	8.8	6.4	4	2.4
		02/20/93	4	7.3	7.3	4.3	3.0
21 DAY	CFB13	01/29/93	-18	4.5	7.2	3.6	3.6
		02/16/93	0	7.8	7.4	3.8	3.6
		02/20/93	4	5.7	6.9	3.7	3.2
		03/02/93	14	6.9	7.2	4.0	3.2
		03/09/93	21	6.7	7.2	4.0	3.2
LONG-TERM	CFB15	02/03/93	-13	5.5	7.2	4	3.2
		02/16/93	0	8.5	7.4	4	3.4
		02/20/93	4	6.8	7.2	4	3.2
		03/02/93	14	6.4	6.8	3.7	3.1
		03/09/93	21	6.4	7.1	4.1	3.0
		04/10/93	53	6.2	6.9	3.9	3.0

NECROPSY	ANIMAL	DATE	DAY	SGOT	SGPT	LDH	ALK.PHOS.
4 DAY	CFB11	02/03/93	-13	14	32	261	1202
		02/16/93	0	18	39	243	937
		02/20/93	4	14	30	241	995
21 DAY	CFB13	01/29/93	-18	26	162	166	641
		02/16/93	0	28	45	227	561
		02/20/93	4	21	33	179	521
		03/02/93	14	31	76	206	725
		03/09/93	21	30	51	229	743
LONG-TERM	CFB15	02/03/93	-13	8	27	152	672
		02/16/93	0	11	31	156	683
		02/20/93	4	9	31	172	649
		03/02/93	14	26	49	217	675
		03/09/93	21	36	52	264	659
		04/10/93	53	17.0	30	210	833

Table IIIf - Blood Chemistries for Toxicity Studies - Study III (Virus = 1.0E+09) (cont.)

NECROPSY	ANIMAL	DATE	DAY	T.BILIRUBIN	CHOLESTEROL		
4 DAY	CFB11	02/03/93	-13	0.1	131		
		02/16/93	0	0.1	131		
		02/20/93	4	0.2	149		
21 DAY	CFB13	01/29/93	-18	0.2	109		
		02/16/93	0	0.2	107		
		02/20/93	4	0.2	100		
		03/02/93	14	0.1	102		
		03/09/93	21	0.2	99		
LONG-TERM	CFB15	02/03/93	-13	0	116		
		02/16/93	0	0.2	128		
		02/20/93	4	0.2	128		
		03/02/93	14	0.1	113		
		03/09/93	21	0.1	113		
		04/10/93	53	0.1	111		

Table IIIg - Blood Chemistries for Toxicity Studies - Study III (Virus = 1.0E+10)

NECROPSY	ANIMAL	DATE	DAY	SODIUM	POTASSIUM	CHLORIDE	HCO3
4 DAY	CFB4	01/18/93	0	145	3.6	100	31
		01/22/93	4	145	3.6	102	30
21 DAY	CFB6	01/18/93	0	149	4.4	102	31
		01/22/93	4	150	4.0	101	24
		02/02/93	15	146	4.3	100	31
		02/08/93	21	149	3.7	103	29
LONG-TERM	CFB8	01/18/93	0	148	3.7	106	25
		01/22/93	4	146	3.9	101	29
		02/02/93	15	148	3.8	104	28
		02/08/93	21	149	3.6	104	23
		03/10/93	51	149	3.8	102	32
		04/10/93	82	147	3.4	106	25
NECROPSY	ANIMAL	DATE	DAY	BUN	CREATININE	GLUCOSE	CALCIUM
4 DAY	CFB4	01/18/93	0	17	0.5	70	10.0
		01/22/93	4	19	0.5	56	10.0
21 DAY	CFB6	01/18/93	0	16	0.9	95	10.2
		01/22/93	4	18	1.0	75	10.1
		02/02/93	15	14	1.0	92	9.4
		02/08/93	21	14	0.8	93	9.6
LONG-TERM	CFB8	01/18/93	0	14	0.7	79	9.9
		01/22/93	4	14	0.6	79	9.7
		02/02/93	15	14	0.8	64	9.5
		02/08/93	21	12	0.8	83	9.3
		03/10/93	51	16	0.8	66	9.3
		04/10/93	82	16	0.7	79	9.5

Table IIIg - Blood Chemistries for Toxicity Studies - Study III (Virus = 1.0E+10) (cont.)

NECROPSY	ANIMAL	DATE	DAY	PHOSPHORUS	T.PROTEIN	ALBUMIN	GLOBULIN
4 DAY	CFB4	01/18/93	0	5.6	6.9	3.6	3.3
		01/22/93	4	5.5	6.7	3.5	3.2
21 DAY	CFB6	01/18/93	0	5.5	7.2	4.1	3.1
		01/22/93	4	5.6	7.4	4.3	3.1
		02/02/93	15	4.6	6.6	3.5	3.1
		02/08/93	21	4.5	6.8	3.8	3.0
LONG-TERM	CFB8	01/18/93	0	6.4	6.5	3.7	2.8
		01/22/93	4	4.7	6.5	3.6	2.9
		02/02/93	15	5.4	6.3	3.4	2.9
		02/08/93	21	5.5	6.2	3.5	2.7
		03/10/93	51	5.5	6.6	3.7	2.9
		04/10/93	82	5.5	6.4	3.6	2.8

NECROPSY	ANIMAL	DATE	DAY	SGOT	SGPT	LDH	ALK.PHOS.
4 DAY	CFB4	01/18/93	0	28	32	216	877
		01/22/93	4	45	34	512	822
21 DAY	CFB6	01/18/93	0	17	28	178	579
		01/22/93	4	23	26	291	503
		02/02/93	15	23	26	253	483
		02/08/93	21	28	33	291	486
LONG-TERM	CFB8	01/18/93	0	32	29	283	782
		01/22/93	4	25	25	312	677
		02/02/93	15	32	24	327	723
		02/08/93	21	31	30	335	724
		03/10/93	51	30	28	321	728
		04/10/93	82	34	27	326	855

Table IIIg - Blood Chemistries for Toxicity Studies - Study III (Virus = 1.0E+10) (cont.)

NECROPSY	ANIMAL	DATE	DAY	T.BILIRUBIN	CHOLESTEROL		
4 DAY	CFB4	01/18/93	0	0.2	97		
		01/22/93	4	0.2	102		
21 DAY	CFB6	01/18/93	0	0.2	79		
		01/22/93	4	0.2	88		
		02/02/93	15	0.2	76		
		02/08/93	21	0.2	81		
LONG-TERM	CFB8	01/18/93	0	0.1	78		
		01/22/93	4	0.1	85		
		02/02/93	15	0.2	78		
		02/08/93	21	0.1	79		
		03/10/93	51	0.1	81		
		04/10/93	82	0.2	82		

Table IVa - Urinalysis for Baboon Toxicity Studies (Study I)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urine	Specific gravity	pH	Leuk esterase
1.0E+10	4 DAY	CFB1	10/20/92	-2	N D	N D	N D	N D
VIRUS	NECROPSY	ANIMAL	DATE	DAY	Nitrite	Protein	Glucose	Ketones
1.0E+10	4 DAY	CFB1	10/20/92	-2	N D	N D	N D	N D
VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urobilin	Bilirubin	Blood	RBC/hpf
1.0E+10	4 DAY	CFB1	10/20/92	-2	N D	N D	N D	N D
VIRUS	NECROPSY	ANIMAL	DATE	DAY	WBC/hpf	Casts		
1.0E+10	4 DAY	CFB1	10/20/92	-2	N D	N D		

Table IVb - Urinalysis for Baboon Toxicity Studies (Study II)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urine	Specific gravity	pH	Leuk esterase
1.0E+10	LONG-TERM	CFB2	11/09/92	0	N D	N D	6	0
			11/12/92	3	YELLOW, CLEAR	1.005	5	0
			11/30/92	21	N D	N D	6	0
			01/25/93	77	N D	N D	6	0
VIRUS	NECROPSY	ANIMAL	DATE	DAY	Nitrite	Protein	Glucose	Ketones
1.0E+10	LONG-TERM	CFB2	11/09/92	0	N D	0	0	0
			11/12/92	3	0	0	0	0
			11/30/92	21	N D	0	0	0
			01/25/93	77	N D	0	0	0
VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urobilin	Bilirubin	Blood	RBC/hpf
1.0E+10	LONG-TERM	CFB2	11/09/92	0	N D	N D	0	N D
			11/12/92	3	0	0	0	occ
			11/30/92	21	N D	N D	0	N D
			01/25/93	77	N D	N D	1	N D
VIRUS	NECROPSY	ANIMAL	DATE	DAY	WBC/hpf	Casts		
1.0E+10	LONG-TERM	CFB2	11/09/92	0	N D	N D		
			11/12/92	3	occ	0		
			11/30/92	21	N D	N D		
			01/25/93	77	N D	N D		

Table IVc - Urinalysis for Baboon Toxicity Studies (Study III)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urine	Specific gravity	pH	Leuk esterase
1.0E+07	4 DAY	CFB3	01/18/93	0	YELLOW, HAZY	1.005	7	0
			01/22/93	4	YELLOW, HAZY	1.005	7	0
	21 DAY	CFB5	01/18/93	0	YELLOW, CLEAR	1.012	8	0
			02/08/93	21	YELLOW, CLEAR	1.005	7	0
	LONG-TERM	CFB7	01/18/93	0	YELLOW, HAZY	1.019	5	0
			01/22/93	4	YELLOW, HAZY	1.005	8	0
			02/02/93	15	YELLOW, CLEAR	1.018	8	0
			02/08/93	21	YELLOW, CLEAR	1.017	8	0

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Nitrite	Protein	Glucose	Ketones
1.0E+07	4 DAY	CFB3	01/18/93	0	0	0	0	0
			01/22/93	4	0	0	0	0
	21 DAY	CFB5	01/18/93	0	0	0	0	0
			02/08/93	21	0	0	0	0
	LONG-TERM	CFB7	01/18/93	0	0	0	0	0
			01/22/93	4	0	0	0	0
			02/02/93	15	0	0	0	0
			02/08/93	21	0	0	0	0

Table IVc - Urinalysis for Baboon Toxicity Studies (Study III) (cont.)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urobilin	Bilirubin	Blood	RBC/hpf
1.0E+07	4 DAY	CFB3	01/18/93	0	0	0	0	0
			01/22/93	4	0	0	0	0
	21 DAY	CFB5	01/18/93	0	0	0	0	ND
			02/08/93	21	0	0	0	ND
	LONG-TERM	CFB7	01/18/93	0	0	0	0	0
			01/22/93	4	0	0	0	ND
			02/02/93	15	0	0	0	ND
			02/08/93	21	0	0	0	ND

VIRUS	NECROPSY	ANIMAL	DATE	DAY	WBC/hpf	Casts
1.0E+07	4 DAY	CFB3	01/18/93	0	0	0
			01/22/93	4	0	0
	21 DAY	CFB5	01/18/93	0	ND	ND
			02/08/93	21	ND	ND
	LONG-TERM	CFB7	01/18/93	0	occ	0
			01/22/93	4	ND	ND
			02/02/93	15	ND	ND
			02/08/93	21	ND	ND

Table IVd - Urinalysis for Baboon Toxicity Studies (Study III)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urine	Specific gravity	pH	Leuk esterase
1.0E+08	4 DAY	CFB10	02/16/93	0	YELLOW, CLEAR	1.004	6	0
			02/20/93	4	YELLOW, CLEAR	1.003	6	0
	21 DAY	CFB14	02/16/93	0	YELLOW, HAZY	1.005	7	0
			02/20/93	4	YELLOW, CLEAR	1.010	5	0
			03/02/93	14	YELLOW, CLEAR	1.003	7	0
			03/09/93	21	YELLOW, CLEAR	1.004	7	0
	LONG-TERM	CFB16	02/16/93	0	STRAW, CLEAR	1.001	5	0
			02/20/93	4	YELLOW, CLOUDY	1.024	5	0
			03/02/93	14	YELLOW, HAZY	1.028	8	0
			03/09/93	21	YELLOW, CLEAR	1.011	8	0

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Nitrite	Protein	Glucose	Ketones
1.0E+08	4 DAY	CFB10	02/16/93	0	0	0	0	0
			02/20/93	4	0	0	0	0
	21 DAY	CFB14	02/16/93	0	0	0	0	0
			02/20/93	4	0	15	0	0
			03/02/93	14	0	0	0	0
			03/09/93	21	0	0	0	0
	LONG-TERM	CFB16	02/16/93	0	0	0	0	0
			02/20/93	4	0	30	0	0
			03/02/93	14	0	15	0	0
			03/09/93	21	0	15	0	0

Table IVd - Urinalysis for Baboon Toxicity Studies (Study III) (cont.)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urobilin	Bilirubin	Blood	RBC/hpf
1.0E+08	4 DAY	CFB10	02/16/93	0	0	0	0	N D
			02/20/93	4	0	0	0	N D
	21 DAY	CFB14	02/16/93	0	0	0	0	N D
			02/20/93	4	0	0	300	0 to 3
			03/02/93	14	0	0	0	N D
			03/09/93	21	0	0	0	N D
	LONG-TERM	CFB16	02/16/93	0	0	0	0	N D
			02/20/93	4	0	0	0	5 to 10
			03/02/93	14	0	0	60	50 to 75
			03/09/93	21	0	0	10	5 to 10

VIRUS	NECROPSY	ANIMAL	DATE	DAY	WBC/hpf	Casts
1.0E+08	4 DAY	CFB10	02/16/93	0	N D	N D
			02/20/93	4	N D	N D
	21 DAY	CFB14	02/16/93	0	N D	N D
			02/20/93	4	occ	0
			03/02/93	14	N D	N D
			03/09/93	21	N D	N D
	LONG-TERM	CFB16	02/16/93	0	N D	N D
			02/20/93	4	0	0
			03/02/93	14	0 to 3	0
			03/09/93	21	0 to 3	0

Table IVe - Urinalysis for Baboon Toxicity Studies (Study III)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urine	Specific gravity	pH	Leuk esterase
1.0E+09	4 DAY	CFB11	02/16/93	0	YELLOW, HAZY	1.020	5	0
			02/20/93	4	YELLOW, CLEAR	1.016	7	0
	21 DAY	CFB13	02/16/93	0	STRAW, CLEAR	1.005	5	0
			02/20/93	4	YELLOW, CLEAR	1.005	7	0
			03/02/93	14	YELLOW, CLOUDY	1.021	8	0
			03/09/93	21	YELLOW, CLEAR	1.005	5	0
	LONG-TERM	CFB15	02/16/93	0	YELLOW, CLEAR	1.020	7	0
			02/20/93	4	YELLOW, CLEAR	1.021	5	0
			03/02/93	14	YELLOW, CLEAR	1.011	7	0
			03/09/93	21	YELLOW, CLEAR	1.020	7	0

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Nitrite	Protein	Glucose	Ketones
1.0E+09	4 DAY	CFB11	02/16/93	0	0	100	0	0
			02/20/93	4	0	15	0	0
	21 DAY	CFB13	02/16/93	0	0	0	0	0
			02/20/93	4	0	0	0	0
			03/02/93	14	0	0	0	0
			03/09/93	21	0	0	0	0
	LONG-TERM	CFB15	02/16/93	0	0	0	0	0
			02/20/93	4	0	30	0	0
			03/02/93	14	0	0	0	0
			03/09/93	21	0	30	0	0

Table IVe - Urinalysis for Baboon Toxicity Studies (Study III) (cont.)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urobilin	Bilirubin	Blood	RBC/hpf
1.0E+09	4 DAY	CFB11	02/16/93	0	0	0	300	75 to 100
			02/20/93	4	0	0	60	5 to 10
	21 DAY	CFB13	02/16/93	0	0	0	0	N D
			02/20/93	4	0	0	0	N D
			03/02/93	14	0	0	0	N D
			03/09/93	21	0	0	0	N D
	LONG-TERM	CFB15	02/16/93	0	0	0	0	N D
			02/20/93	4	0	0	0	occ
			03/02/93	14	0	0	0	N D
			03/09/93	21	0	0	10	3 to 5

VIRUS	NECROPSY	ANIMAL	DATE	DAY	WBC/hpf	Casts
1.0E+09	4 DAY	CFB11	02/16/93	0	3 to 5	0
			02/20/93	4	0 to 3	0
	21 DAY	CFB13	02/16/93	0	N D	N D
			02/20/93	4	N D	N D
			03/02/93	14	N D	N D
			03/09/93	21	N D	N D
	LONG-TERM	CFB15	02/16/93	0	N D	N D
			02/20/93	4	0	0
			03/02/93	14	N D	N D
			03/09/93	21	0	0

Table IVf - Urinalysis for Baboon Toxicity Studies (Study III)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urine	Specific gravity	pH	Leuk esterase
1.0E+10	4 DAY	CFB4	01/18/93	0	YELLOW, CLEAR	1.015	8	0
			01/22/93	4	YELLOW, HAZY	1.003	8	0
	21 DAY	CFB6	01/18/93	0	YELLOW, CLOUDY	1.023	8	0
			02/02/93	15	YELLOW, HAZY	1.017	7	0
			02/08/93	21	YELLOW, CLEAR	1.013	8	0
	LONG-TERM	CFB8	01/22/93	4	YELLOW, CLEAR	1.008	8	0
			02/02/93	21	YELLOW, HAZY	1.018	7	0

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Nitrite	Protein	Glucose	Ketones
1.0E+10	4 DAY	CFB4	01/18/93	0	0	0	0	0
			01/22/93	4	0	0	0	0
	21 DAY	CFB6	01/18/93	0	0	0	0	0
			02/02/93	15	0	30	0	0
			02/08/93	21	0	0	0	0
	LONG-TERM	CFB8	01/22/93	4	0	0	0	0
			02/02/93	21	0	15	0	0

Table IVf - Urinalysis for Baboon Toxicity Studies (Study III) (cont.)

VIRUS	NECROPSY	ANIMAL	DATE	DAY	Urobilin	Bilirubin	Blood	RBC/hpf
1.0E+10	4 DAY	CFB4	01/18/93	0	0	0	0	0
			01/22/93	4	0	0	0	N D
	21 DAY	CFB6	01/18/93	0	0	0	0	10 to 25
			02/02/93	15	0	0	0	occ
			02/08/93	21	0	0	0	N D
	LONG-TERM	CFB8	01/22/93	4	0	0	0	N D
			02/02/93	21	0	0	0	occ

VIRUS	NECROPSY	ANIMAL	DATE	DAY	WBC/hpf	Casts
1.0E+10	4 DAY	CFB4	01/18/93	0	occ	0
			01/22/93	4	N D	N D
	21 DAY	CFB6	01/18/93	0	occ	0
			02/02/93	15	3 to 5	0
			02/08/93	21	N D	N D
	LONG-TERM	CFB8	01/22/93	4	N D	N D
			02/02/93	21	occ	0

Table V- Arterial Blood Gases for Baboon Toxicity Studies

	VIRUS	NECROPSY	ANIMAL	DATE	DAY	pH	PCO_2	PO_2	HCO_3 calc	P(A-a)O_2	Δ P(A-a)O_2
NORMAL ADULT BABOON						N/A	N/A	N/A	N/A	N/A	
NORMAL JUVENILE BABOON											
STUDY I	1.0E+10	4 DAY	CFB1	10/20/92	-2	7.39	55.4	54.0	33.9	26.2	
				10/20/92	-2	7.39	54.6	58.0	33.5	22.4	
				10/22/92	0	7.39	54.7	54.0			
STUDY II	1.0E+10	LONG-TERM	CFB2	11/06/92	-3	7.38	45.0	62.0	27.0	30.3	3.7
				11/09/92	0	7.35	45.0	63.0	26.9	26.6	0.0
				11/12/92	3	7.34	51.0	54.0	28.1	27.4	0.8
				11/23/92	14	7.39	46.0	58.0	27.8	31.3	4.7
				11/30/92	21	7.36	52.0	44.0	29.2	39.2	12.6
				02/10/93	93	7.38	47.5	56.0	28.5	33.1	6.5
				03/05/93	116	7.38	48.5	63.0	29.0	22.7	-3.9
				04/10/93	152	7.34	51.6	47.0	28.4	35.4	8.8
STUDY III	1.0E+07	4 DAY	CFB3	01/18/93	0	7.39	49.0	89.0	30.1	-3.3	0.0
				01/22/93	4	7.40	48.2	86.0	30.4	0.9	4.2
		21 DAY	CFB5	01/18/93	0	7.40	47.0	74.0	29.6	14.7	0.0
				01/22/93	4	7.40	46.6	63.0	29.4	23.4	8.7
				02/02/93	15	7.38	49.0	86.0	29.5	0.4	-14.3
				02/08/93	21	7.41	46.2	104.0	30.0	15.6	0.9
	VIRUS	NECROPSY	ANIMAL	DATE	DAY	pH	PCO	PO	HCO calc	P(A-a)O	P(A-a)O

Table V- Arterial Blood Gases for Baboon Toxicity Studies

STUDY III		LONG-TERM	CFB7	01/18/93	0	7.41	41.8	82.0	27.0	13.4	0.0
				01/22/93	4	7.41	51.0	63.0	32.6	20.5	7.1
				02/02/93	15	7.37	49.3	39.0	28.7	46.6	33.2
				02/08/93	21	7.39	49.8	67.0	30.4	20.1	6.7
				03/10/93	51	7.38	51.8	67.0	31.2	14.9	1.5
				04/10/93	82	7.42	46.5	83.0	30.1	6.1	-7.3
	1.0E+08	4 DAY	CFB10	02/03/93	-13	7.29	41.2	84.0	19.8	14.5	0.0
				02/10/93	-6	7.33	43.3	64	28.2	9.2	-5.3
				02/16/93	0	7.39	46.3	80.0	28.2	9.2	-5.3
				02/20/93	4	7.38	46.1	78.0	27.3	12.1	-2.4
		21 DAY	CFB14	01/29/93	-18	7.37	43.8	76.0	25.6	16.7	2.0
				02/16/93	0	7.40	44.1	77.0	27.3	14.7	0.0
				02/20/93	4	7.38	44.3	83.0	26.5	9.4	-5.3
				03/02/93	14	7.35	43.8	72.0	24.5	19.8	5.1
				03/09/93	21	7.37	47.3	67.0	27.4	21.7	7.0
		LONG-TERM	CFB16	01/29/93	-18	7.28	34.9	89.0	16.6	14.8	-4.5
				02/16/93	0	7.37	42.1	76	24.6	19.3	0.0
				02/20/93	4	7.37	41.6	80	24.3	15.9	-3.4
				03/02/93	14	7.42	38.9	78	25.5	20.1	0.8
				03/09/93	21	7.40	39.7	87	25.1	11.2	-8.1
				04/10/93	53	7.41	40.9	76	25.9	19.7	0.4
	1.0E+09	4 DAY	CFB11	02/03/93	-13	7.20	42.0	84.0	16.6	12.7	6.6
				02/16/93	0	7.15	39.9	90.0	14.3	6.1	0.0
				02/20/93	4	7.32	46.6	67.0	24.4	22.4	16.3
	VIRUS	NECROPSY	ANIMAL	DATE	DAY	pH	PCO	PO	HCO calc	P(A-a)O	P(A-a)O

Table V- Arterial Blood Gases for Baboon Toxicity Studies

STUDY III		**21 DAY**	**CFB13**	01/29/93	-18	7.41	42.6	83.0	27.2	11.2	1.9
				02/16/93	0	7.38	43.7	83.0	26.4	9.3	0.0
				02/20/93	4	7.37	46.0	74.0	27.2	16.2	6.9
				03/02/93	14	7.38	44.4	82.0	26.8	9.5	0.2
				03/09/93	21	7.40	47.5	80.0	29.5	8.0	-1.3
		LONG-TERM	**CFB15**	02/03/93	-13	7.30	41.1	87.0	21.4	10.5	6.5
				02/16/93	0	7.28	36.7	98.0	17.4	4.0	0.0
				02/20/93	4	7.29	38.4	83.0	18.5	16.6	12.6
				03/02/93	14	7.39	40.8	80.0	24.9	15.8	11.8
				03/09/93	21	7.32	37.7	91.0	19.6	8.9	4.9
				04/10/93	53	7.37	40.2	84.0	23.2	12.6	8.6
	1.0E+10	**4 DAY**	**CFB4**	01/18/93	0	7.40	47.0	78.0	29.3	10.9	0.0
				01/22/93	4	Specimen	Clotted				
		21 DAY	**CFB6**	01/18/93	0	7.41	45.3	72.0	29.1	18.8	0.0
				01/22/93	4	7.35	42.9	59.0	23.7	34.0	15.2
				02/02/93	15	7.40	47.1	66.0	29.4	22.8	4.0
				02/08/93	21	7.38	48.5	59.0	28.8	29.0	10.2
		LONG-TERM	**CFB8**	01/18/93	0	7.35	44.0	78.0	24.8	15.0	0.0
				01/22/93	4	7.42	42.6	66.0	28.2	27.0	12.0
				02/02/93	15	7.41	41.6	77.0	26.3	18.2	3.2
				02/08/93	21	7.34	43.2	70.0	23.5	25.0	10.0
				03/10/93	51	7.40	47.4	51.0	29.9	36.5	21.5
				03/15/93	56	7.38	43.0	63.0	25.7	32.4	17.4
				03/24/93	65	7.36	45.4	70.0	25.9	21.1	6.1
				04/10/93	82	7.36	43.7	64.0	24.9	27.7	12.7

Table VI - Chest Radiographs for Baboon Toxicity Studies

	VIRAL DOS	NECROPSY	ANIMAL	DATE	DAY	CHEST RADIOGRAPHIC FINDINGS
STUDY I	1.0E+10	4 DAY	CFB1	10/20/92	-2	RUL and RML atelectasis
STUDY II	1.0E+10	LONG-TERM	CFB2	11/06/92	-3	Normal
				11/09/92	0	±RUL and mild LUL densities
				11/12/92	3	mild-moderate RUL and mild LUL densities
				11/23/92	14	mild RUL and mild LUL densities
				11/30/92	21	normal RUL, moderate LUL density
				01/21/93	73	Normal
				01/25/93	77	Normal
				02/10/93	93	Normal
				03/05/93	116	Normal
				04/10/93	152	Normal
STUDY III	1.0E+07	4 DAY	CFB3	01/18/93	0	Normal
				01/22/93	4	Normal
		21 DAY	CFB5	01/18/93	0	Normal
				01/22/93	4	Normal
				02/02/93	15	Normal
				02/08/93	21	Normal
		LONG-TERM	CFB7	01/18/93	0	linear streaking of medial aspect of lower lobes bilaterally
				01/22/93	4	linear streaking of medial aspect of lower lobes bilaterally
				02/02/93	15	linear streaking of medial aspect of lower lobes bilaterally

Table VI - Chest Radiographs for Baboon Toxicity Studies

	VIRAL DOS	NECROPSY	ANIMAL	DATE	DAY	CHEST RADIOGRAPHIC FINDINGS
				02/08/93	21	linear streaking of medial aspect of lower lobes bilaterally
				03/10/93	51	bilateral medial lower lobes with prominent markings
				04/10/93	82	Normal
STUDY III	1.0E+08	4 DAY	CFB10	02/03/93	-13	ND
				02/10/93	-6	ND
				02/16/93	0	Normal
				02/20/93	4	Normal
		21 DAY	CFB14	01/03/93	-44	Normal
				01/29/93	-18	ND
				02/16/93	0	blurred L hemi-diaphragm on A-P (heart overlying), Lateral is normal
				02/20/93	4	blurred L hemi-diaphragm on A-P, Lateral is normal, improved
				03/02/93	14	blurred L hemi-diaphragm on A-P, Lateral is normal, improved
				03/09/93	21	Normal
		LONG-TERM	CFB16	01/03/93	-44	Normal
				01/29/93	-18	Normal
				02/16/93	0	Normal
				02/20/93	4	Normal
				03/09/93	21	Normal
				04/10/93	53	Normal
	1.0E+09	4 DAY	CFB11	02/16/93	0	Normal

Table VI - Chest Radiographs for Baboon Toxicity Studies

	VIRAL DOS	NECROPSY	ANIMAL	DATE	DAY	CHEST RADIOGRAPHIC FINDINGS
				02/20/93	4	Normal
		21 DAY	CFB13	01/03/93	-44	very mild RLL density
				02/16/93	0	Normal
				02/20/93	4	Normal
				03/02/93	14	Normal
				03/09/93	21	Normal
STUDY III	1.0E+09	LONG-TERM	CFB15	01/03/93	-44	mild LUL streaking, bilateral medial lower lobes with prominent markings
				02/16/93	0	mild LUL streaking, bilateral medial lower lobes with prominent markings
				02/20/93	4	mild LUL streaking, bilateral medial lower lobes with prominent markings
				03/02/93	14	Normal
				03/09/93	21	Normal
				04/10/93	53	Normal
	1.0E+10	4 DAY	CFB4	01/18/93	0	Normal
				01/22/93	4	Normal
		21 DAY	CFB6	01/18/93	0	Normal
				01/22/93	4	± RUL density
				02/02/93	15	severe RUL, moderate LUL, and ± RLL density
				02/08/93	21	moderately severe RUL, mild LUL, and moderate RLL densities
		LONG-TERM	CFB8	01/18/93	0	Normal

Table VI - Chest Radiographs for Baboon Toxicity Studies

	VIRAL DOS	NECROPSY	ANIMAL	DATE	DAY	CHEST RADIOGRAPHIC FINDINGS
				01/22/93	4	mild LUL density
				02/02/93	15	mild-moderate RUL and improved LUL densities
				02/08/93	21	moderate RUL and improved LUL densities
				02/18/93	31	normal RUL, mild LUL
				02/26/93	39	Normal
				03/10/93	51	Normal
				03/15/93	56	moderate density in region of RUL/RML
				03/24/93	65	Normal
				04/10/93	82	Normal

Table VII - Analysis of Bronchoalveolar Lavage

	VIRUS	NECROPSY	ANIMAL	DAY	LOCATION	TOTAL CELL COUNT	% X-Gal	% CFTR	DIFFERENTIAL COUNT			
									% Macro	% PMN	%Eos	% Lymph
NORMAL HUMAN									84-99	0.1-4.4	N/A	0.7-14.4
NORMAL SIMIAN									87.8-96.2	0-2	0-0.5	2.8-11.2
STUDY II	1.0E+10	LONG-TERM	CFB2	0	Right Middle Lobe	4.20E+06	0	0	43	45	7	4
				3	LacZ Segment	2.50E+06	5.2	0	58	14	5	22
				3	CFTR Segment	4.00E+06	0.3	3.4	38	52	2	7
				21	LacZ Segment	3.60E+06	0	ND	73	7	8	12
				21	CFTR Segment	2.90E+06	0	ND	46	5	16	33
STUDY III	1.0E+07	LONG-TERM	CFB7	0	Right Middle Lobe	2.00E+06	0	ND	87	2	1	11
				4	LacZ Segment	2.66E+06	0	ND	54	33	2	11
				4	CFTR Segment	2.25E+06	0	ND	80	7	5	8
				21	LacZ Segment	2.50E+06	0	ND	60	1	1	38
				21	CFTR Segment	3.80E+06	0	ND	83	2	2	13
	1.0E+08	LONG-TERM	CFB16	0	Right Middle Lobe	2.20E+06	0	ND	48	2	1	49
				4	LacZ Segment	2.00E+06	0	ND	67	3	1	29
				4	CFTR Segment	1.80E+06	0	ND	55	3	2	40
				21	LacZ Segment	1.00E+06	0	ND	31	0	3	66
				21	CFTR Segment	7.00E+05	0	ND	62	0	5	33
	1.0E+09	LONG-TERM	CFB15	0	Right Middle Lobe	1.50E+06	0	ND	68	5	5	22
				4	LacZ Segment	3.00E+06	0	ND	60	29	8	4
				4	CFTR Segment	2.50E+06	0	ND	48	34	12	7
				21	LacZ Segment	5.00E+05	0	ND	41	0	18	41
				21	CFTR Segment	4.00E+05	0	ND	50	0	5	45
	1.0E+10	LONG-TERM	CFB8	0	Right Middle Lobe	1.70E+06	0	ND	73	3	9	15

Table VII - Analysis of Bronchoalveolar Lavage

	VIRUS	Total Counted	Macrophages	PMN	EOS	Lymph	Epith	tot cell-epith	DIFFERENTIAL COUNT				
									% Macro	% PMN	%Eos	% Lymph	% Epith
STUDY II	1.0E+10	443							41.8	44.0	6.8	4.3	3.2
		1017							53.7	13.1	4.9	20.1	8.3
		1276							36.9	50.7	2.4	6.8	3.2
		522							63.4	5.9	6.7	10.5	13.4
		532							42.1	4.7	14.8	30.6	7.7
STUDY III	1.0E+07	600	509	11	4	64	12		84.8	1.8	0.7	10.7	2.0
		600	302	183	11	64	40		50.3	30.5	1.8	10.7	6.7
		600	440	39	27	44	50		73.3	6.5	4.5	7.3	8.3
		512	280	6	4	174	48		54.7	1.2	0.8	34.0	9.4
		551	424	8	10	67	45		77.0	1.5	1.8	12.2	8.2
	1.0E+08	562	253	12	4	260	34	528					
		604	394	18	8	170	14	590					
		520	267	15	11	197	33	487					
		634	190	1	16	410	17	617					
		533	318	2	25	168	20	513					
	1.0E+09	500	330	25	24	109	13	487					
		597	356	170	46	23	3	594					
		555	263	190	64	36	3	552					
		633	253	0	111	254	15	618					
		597	242	1	24	221	109	488					
	1.0E+10	600	434	17	55	87	7		72.3	2.8	9.2	14.5	1.2

Table VII - Analysis of Bronchoalveolar Lavage

				4	LacZ Segment	2.45E+06	1.5	ND	22	13	55	10
				4	CFTR Segment	2.25E+06	0	ND	50	4	16	29
				21	LacZ Segment	1.80E+06	0	ND	39	2	10	48
				21	CFTR Segment	2.50E+06	0	ND	28	2	53	16

Table VII - Analysis of Bronchoalveolar Lavage

		600	134	75	325	62	4		22.3	12.5	54.2	10.3	0.7
		600	289	25	95	170	21		48.2	4.2	15.8	28.3	3.5
		674	246	15	64	304	45		36.5	2.2	9.5	45.1	6.7
		554	156	13	294	90	1		28.2	2.3	53.1	16.2	0.2

Table VIII - Analysis of Bronchial Brushings (cont.)

	VIRUS	NECROPSY	ANIMAL	DAY	LOCATION	TOTAL CELL COUNT	% X-Gal	% CFTR
STUDY I	1.0E+10	4 DAY	CFB1	ND				
STUDY II	1.0E+10	LONG-TERM	CFB2	0	Right Middle Lobe	2.4E+06	0	0
				3	LacZ Segment	6.2E+05	0	0
				3	CFTR Segment	5.6E+05	0	2.1
				21	LacZ Segment	3.6E+06	0	ND
				21	CFTR Segment	3.0E+06	0	ND
STUDY III	1.0E+07	4 DAY	CFB3	ND				
		21 DAY	CFB5	ND				
		LONG-TERM	CFB7	0	Left Lower Lobe	1.0E+06	0	0
				4	LacZ Segment	1.0E+06	0	0
				4	CFTR Segment	1.0E+06	0	0
				21	LacZ Segment	8.5E+05	0	0
				21	CFTR Segment	6.9E+05	0	0
	1.0E+08	4 DAY	CFB10	ND				
		21 DAY	CFB14	ND				
		LONG-TERM	CFB16	0	Left Lower Lobe	1.0E+06	0	0

Table VIII - Analysis of Bronchial Brushings (cont.)

	VIRUS	NECROPSY	ANIMAL	DAY	LOCATION	TOTAL CELL COUNT	% X-Gal	% CFTR
				4	LacZ Segment	9.2E+05	0	0
				4	CFTR Segment	1.2E+06	0	0
				21	LacZ Segment	1.2E+06	0	0
				21	CFTR Segment	2.0E+06	0	0
	1.0E+09	4 DAY	CFB11	ND				
		21 DAY	CFB13	ND				
		LONG-TERM	CFB15	0	Left Lower Lobe	1.6E+06	0	0
				4	LacZ Segment	1.2E+06	0	0
				4	CFTR Segment	8.9E+05	0	0
				21	LacZ Segment	1.5E+06	0	0
				21	CFTR Segment	1.8E+06	0	0
	1.0E+10	4 DAY	CFB4	ND				
		21 DAY	CFB6	ND				
		LONG-TERM	CFB8	0	Left Lower Lobe	1.2E+06	0	0
				4	LacZ Segment	1.0E+06	5	0
				4	CFTR Segment	1.3E+06	0	2.5

Table VIII - Analysis of Bronchial Brushings (cont.)

	VIRUS	NECROPSY	ANIMAL	DAY	LOCATION	TOTAL CELL COUNT	% X-Gal	% CFTR
				21	LacZ Segment	1.3E+06	0	0
				21	CFTR Segment	1.2E+06	0	0

Table IX - LacZ Adenovirus Assay Results

	VIRUS	NECROPSY	ANIMAL	DAY	X - GAL						
					(Scale: -, ±, +, ++, +++)						
					BODY FLUID TYPE						
					NASAL	BLOOD	URINE	RECTAL	LAVAGE		
									MIDDLE LOBE	LacZ SEGMENT	CFTR SEGMENT
STUDY I	1.0E+10	4 DAY	CFB1	-							
STUDY II	1.0E+10	LONG-TERM	CFB2	0	-	-	-	-	-		
				3	-	-	-	-		+	
				14	-	-		-		-	
				21	-	-		-		-	
STUDY III	1.0E+07	4 DAY	CFB3	0	-	-	-	-			
				4	-	-	-	-			
		21 DAY	CFB5	0	-	-		-			
				4	-	-		-			
				15	-	-	-	-			
				21	-	-	-	-			
		LONG-TERM	CFB7	0	-	-	-	-	-		
				4	-	-	-	-		-	-
				15	-	-	-	-			
				21	-	-	-	-		-	-
	1.0E+08	4 DAY	CFB10	0	-	-	-	-			
				4	-	-	-	-			

Table IX - LacZ Adenovirus Assay Results

	VIRUS	NECROPSY	ANIMAL	DAY	X - GAL						
					(Scale: -, ±, +, ++, +++)						
					BODY FLUID TYPE						
					NASAL	BLOOD	URINE	RECTAL	LAVAGE		
									MIDDLE LOBE	LacZ SEGMENT	CFTR SEGMENT
		21 DAY	CFB14	0	-	-	-	-			
				4	-	-	-	-			
		LONG-TERM	CFB16	0	-	-	-	-	-		
				4	-	-	-	-		-	-
STUDY III	1.0E+09	4 DAY	CFB11	0	-	-	-	-			
				4	-	-	-	-			
		21 DAY	CFB13	0	-	-	-	-			
				4	-	-	-	-			
		LONG-TERM	CFB15	0	-	-	-	-	-		
				4	-	-	-	-		-	-
	1.0E+10	4 DAY	CFB4	0	-	-	-	-			
				4	-	-	-	-			
		21 DAY	CFB6	0	-	-	-	-			
				4	-	-		-			
				15	-	-	-	-			
				21	-	-	-	-			

Table IX - LacZ Adenovirus Assay Results

	VIRUS	NECROPSY	ANIMAL	DAY	X - GAL						
					(Scale: -, ±, +, ++, +++)						
					BODY FLUID TYPE						
					NASAL	BLOOD	URINE	RECTAL	LAVAGE		
									MIDDLE LOBE	LacZ SEGMENT	CFTR SEGMENT
		LONG-TERM	CFB8	0	-	-		-	-		
				4	-	-	-	-		-	-
				15	-	-		-			
				21	-	-	-	-		-	-

Table IX Addendum - LacZ Adenovirus Positive Control Study Results

DATE		DAY	X-GAL (Scale: -, ±, +, ++, +++) Particles per Plate					
			10,000	1,000	100	10	1	0
CFB3-CFB8								
01/17/89		0	+++	++	+	±	-	-
01/21/89		4	+++	++	+	±	-	-
02/01/89		15	ND	++	+	±	-	-
02/07/89		21	ND	ND	±	-	-	-
CFB10-CFB16								
02/15/89		0	ND	+	+	±	-	-

Table X - General Adenovirus Assay Results

	VIRUS	NECROPSY	ANIMAL	DAY	BODY FLUID AMOUNT	CYTOPATHIC EFFECT						
									(Scale: -, ±, +)			
						NASAL	BLOOD	URINE	RECTA	LAVAGE		
										RIGHT MIDDLE LOBE	LacZ SEGMENT	CFTR SEGMENT
STUDY I	1.0E+10	4 DAY	CFB1	-	N D							
STUDY II	1.0E+10	LONG-TERM	CFB2	-	N D							
STUDY III	1.0E+07	4 DAY	CFB3	0	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
				4	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
		21 DAY	CFB5	0	10 µl of body fluid	-	-		-			
					100 µl of body fluid	-	-		-			
				4	10 µl of body fluid	-	-		-			
					100 µl of body fluid	-	-		-			
				15	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
				21	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
		LONG-TERM	CFB7	0	10 µl of body fluid	-	-	-	-	-		

Table X - General Adenovirus Assay Results

	VIRUS	NECROPSY	ANIMAL	DAY	BODY FLUID AMOUNT			CYTOPATHIC EFFECT				
									(Scale: -, ±, +)			
						NASAL	BLOOD	URINE	RECTA	LAVAGE		
										RIGHT MIDDLE LOBE	LacZ SEGMENT	CFTR SEGMENT
					100 µl of body fluid	-	-	-	-	-		
				4	10 µl of body fluid	-	-	-	-		-	-
					100 µl of body fluid	-	-	-	-		-	-
				15	10 µl of body fluid	-	-	-	-			
STUDY III					100 µl of body fluid	-	-	-	-			
				21	10 µl of body fluid	-	-	-	-		-	-
					100 µl of body fluid	-	-	-	-		-	-
	1.0E+08	4 DAY	CFB10	0	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
				4	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
		21 DAY	CFB14	0	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
				4	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
		LONG-TERM	CFB16	0	10 µl of body fluid	-	-	-	-	-		

Table X - General Adenovirus Assay Results

	VIRUS	NECROPSY	ANIMAL	DAY	BODY FLUID AMOUNT			CYTOPATHIC EFFECT				
									(Scale: -, ±, +)			
						NASAL	BLOOD	URINE	RECTA	LAVAGE		
										RIGHT MIDDLE LOBE	LacZ SEGMENT	CFTR SEGMENT
					100 µl of body fluid	-	-	-	-	-		
				4	10 µl of body fluid	-	-	-	-		-	-
					100 µl of body fluid	-	-	-	-		-	-
	1.0E+09	4 DAY	CFB11	0	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
				4	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
STUDY III		21 DAY	CFB13	0	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
				4	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
		LONG-TERM	CFB15	0	10 µl of body fluid	-	-	-	-	-		
					100 µl of body fluid	-	-	-	-	-		
				4	10 µl of body fluid	-	-	-	-		-	-
					100 µl of body fluid	-	-	-	-		-	-
	1.0E+10	4 DAY	CFB4	0	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			

Table X - General Adenovirus Assay Results

	VIRUS	NECROPSY	ANIMAL	DAY	BODY FLUID AMOUNT			CYTOPATHIC EFFECT				
									(Scale: -, ±, +)			
						NASAL	BLOOD	URINE	RECTA	LAVAGE		
										RIGHT MIDDLE LOBE	LacZ SEGMENT	CFTR SEGMENT
				4	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
		21 DAY	CFB6	0	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
				4	10 µl of body fluid	-	-		-			
					100 µl of body fluid	-	-		-			
				15	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
				21	10 µl of body fluid	-	-	-	-			
					100 µl of body fluid	-	-	-	-			
STUDY III		LONG-TERM	CFB8	0	10 µl of body fluid	-	-		-	-		
					100 µl of body fluid	-	-		-	-		
				4	10 µl of body fluid	-	-	-	-		-	-
					100 µl of body fluid	-	-	-	-		-	-
				15	10 µl of body fluid	-	-		-			
					100 µl of body fluid	-	-		-			
				21	10 µl of body fluid	-	-	-	-		-	-

Table X - General Adenovirus Assay Results

	VIRUS	NECROPSY	ANIMAL	DAY	BODY FLUID AMOUNT			CYTOPATHIC EFFECT				
									(Scale: -, ±, +)			
						NASAL	BLOOD	URINE	RECTA	LAVAGE		
										RIGHT MIDDLE LOBE	LacZ SEGMENT	CFTR SEGMENT
					100 µl of body fluid	-	-	-	-		-	-

Table X Addendum - General Adenovirus Positive Control Results

DATE	DAY	CYTOPATHIC EFFECT (Scale: -, ±, +; P = pending)					
		Particles per Plate					
		10,000	1,000	100	10	1	0
CFB3-CFB8							
1/17/89	0	+	+	+	±	±	-
1/21/89	4	+	+	+	±	±	-
2/1/89	15		+	+	±	±	-
2/7/89	21		+	±	±	-	-
CFB10-CFB16							
2/15/89	0		+	±	±	-	-

WE CLAIM:

1. A vector comprising an adenoviral component comprising at least a portion of an adenoviral genome, the adenoviral component further comprising at least a portion of the adenoviral E3 region and a deletion of at least a portion of the adenoviral E1 region located upstream from the E3 region, wherein the adenoviral component further includes a mutation which results in at least one of the following effects:

a) reduction in expressed or over-expressed adenoviral protein; or

b) reduced viral replication.

2. The vector of Claim 1, wherein the mutation is a temperature-sensitive mutation.

3. The vector of Claim 1, wherein the mutation of the adenoviral component comprises a deletion in a gene for an adenoviral protein and a deletion in a gene for a product which facilitates viral replication.

4. The vector of Claim 2, wherein the mutation of the adenoviral component comprises a deletion in a gene for an adenoviral protein.

5. The vector of Claim 2, wherein the mutation of the adenoviral component comprises a deletion in a gene for a product which facilitates adenoviral replication.

6. A vector comprising an adenoviral component comprising at least a portion of an adenoviral genome, the adenoviral component further comprising at least a portion of the adenoviral E3 region, a first deletion of at least a portion of the adenoviral E1 region located upstream from the E3 region, and a second deletion in an adenoviral region other than E1 and E3.

7. The vector of Claim 6, wherein the second deletion comprises at least a portion of the E2a region.

8. The vector of Claim 6, further comprising a transgene operatively-linked thereto.

9. The vector of Claim 6, wherein the transgene comprises the gene for cystic fibrosis transmembrane regulator (CFTR).

10. The vector of Claim 8, further comprising a promoter operatively-linked to the transgene.

11. A vector comprising an adenoviral component comprising at least a portion of an adenoviral genome, the adenoviral component further comprising at least a portion of the adenoviral E3 region and a deletion of at least a portion of the adenoviral E1 region located upstream from the E3 region, and the transgene for cystic fibrosis transmembrane regulator (CFTR) operatively-linked thereto.

12. The vector of Claim 11, further comprising a promoter operatively-linked to the transgene.

13. A vector comprising an adenoviral component comprising at least a portion of an adenoviral genome, the adenoviral component further comprising at least a portion of the adenoviral E3 region and a first deletion of at least a portion of the adenoviral E1 region located upstream from the E3 region, wherein the adenoviral component further includes a mutation which results in at least one of the following effects:

a) reduction in expressed or over-expressed adenoviral protein; or

b) reduced viral replication,

and wherein the adenoviral component further comprises a second deletion in an adenoviral region other than E1 and E3.

14. The vector of Claim 13, further comprising a transgene operatively-linked thereto, and wherein one of the following effects further includes:

c) increased persistence of transgene expression.

15. The vector of Claim 13, wherein the mutation is a temperature-sensitive mutation.

16. The vector of Claim 14, wherein the transgene comprises the gene for cystic fibrosis transmembrane regulator (CFTR).

17. A method of treatment of a defect in a gene in a target cell or tissue comprising:

a) providing an adenovirus vector comprising at least a portion of the adenoviral E3 region, a first deletion of at least a portion of the adenoviral E1 region located upstream of the E1 region, a second deletion in an adenoviral region other than E1 and E3, and a transgene operatively-linked thereto; and

b) transferring a therapeutically effective amount of the adenovirus vector into the target cell or tissue.

18. The method of Claim 17, wherein the vector further comprises an adenoviral mutation which results in at least one of the following effects:

a) increased persistence of transgene expression;

b) reduction in expressed or over-expressed adenoviral protein; or

c) reduced viral replication.

19. The method of Claim 17, wherein the transgene comprises the gene for cystic fibrosis transmembrane regulator (CFTR).

20. The method of Claim 18, wherein the mutation is a temperature-sensitive mutation.

21. A vector comprising an adenoviral component comprising at least a portion of an adenoviral genome, the adenoviral component further comprising at least a portion of the adenoviral E3 region, a first deletion of at least a portion of the adenoviral E1 region located upstream from the E3 region, wherein the adenoviral component further includes a mutation which results in at least one of the following effects:

a) reduction in expressed or over-expressed adenoviral protein; or

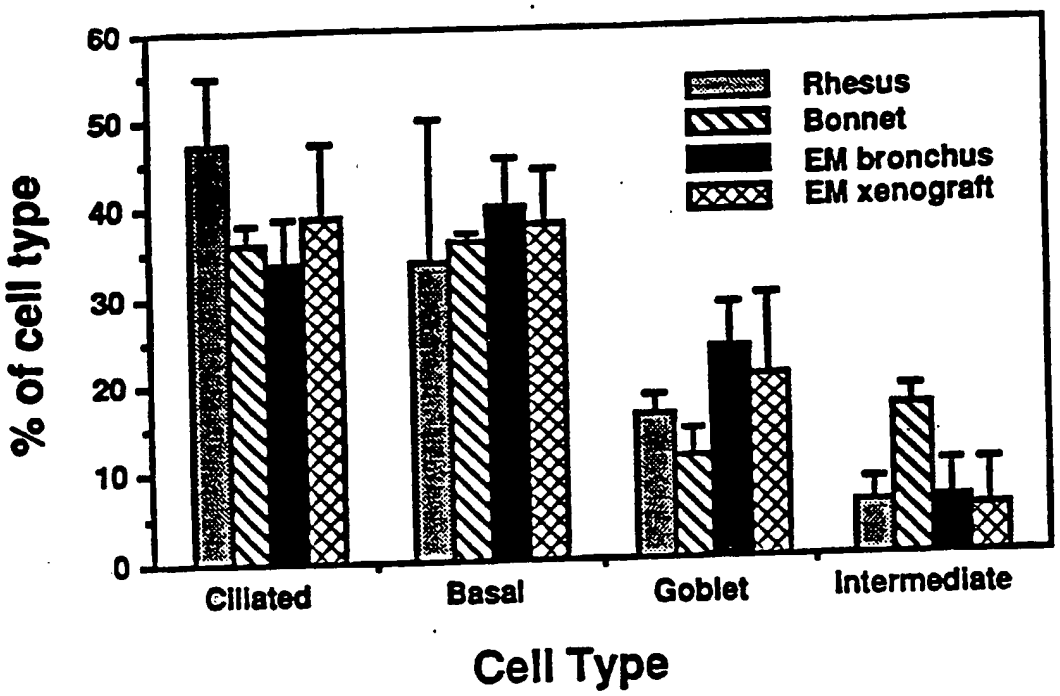
b) reduced viral replication,

and wherein the adenoviral component further comprises a second deletion in an adenoviral region other than E1 and E3, the vector further comprising a transgene operatively-linked to the adenorviral component.

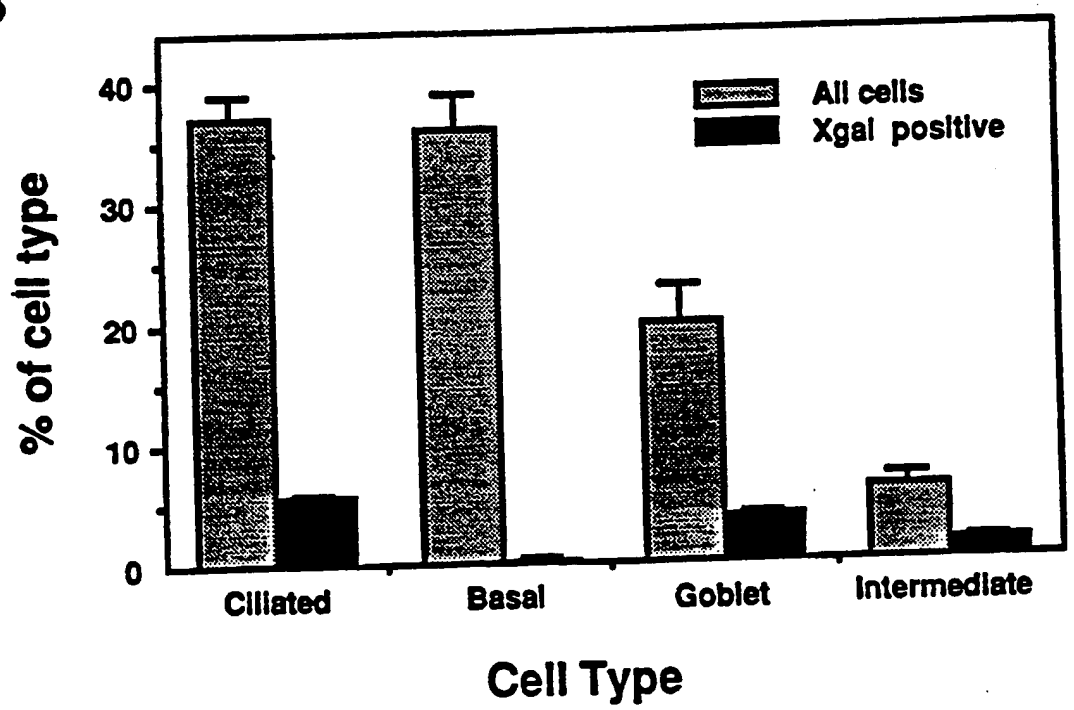
22. The vector of Claim 21, wherein the mutation is a temperature-sensitive mutation.

23. The vector of Claim 21, wherein the transgene comprises the gene for cystic fibrosis transmembrane regulator (CFTR).

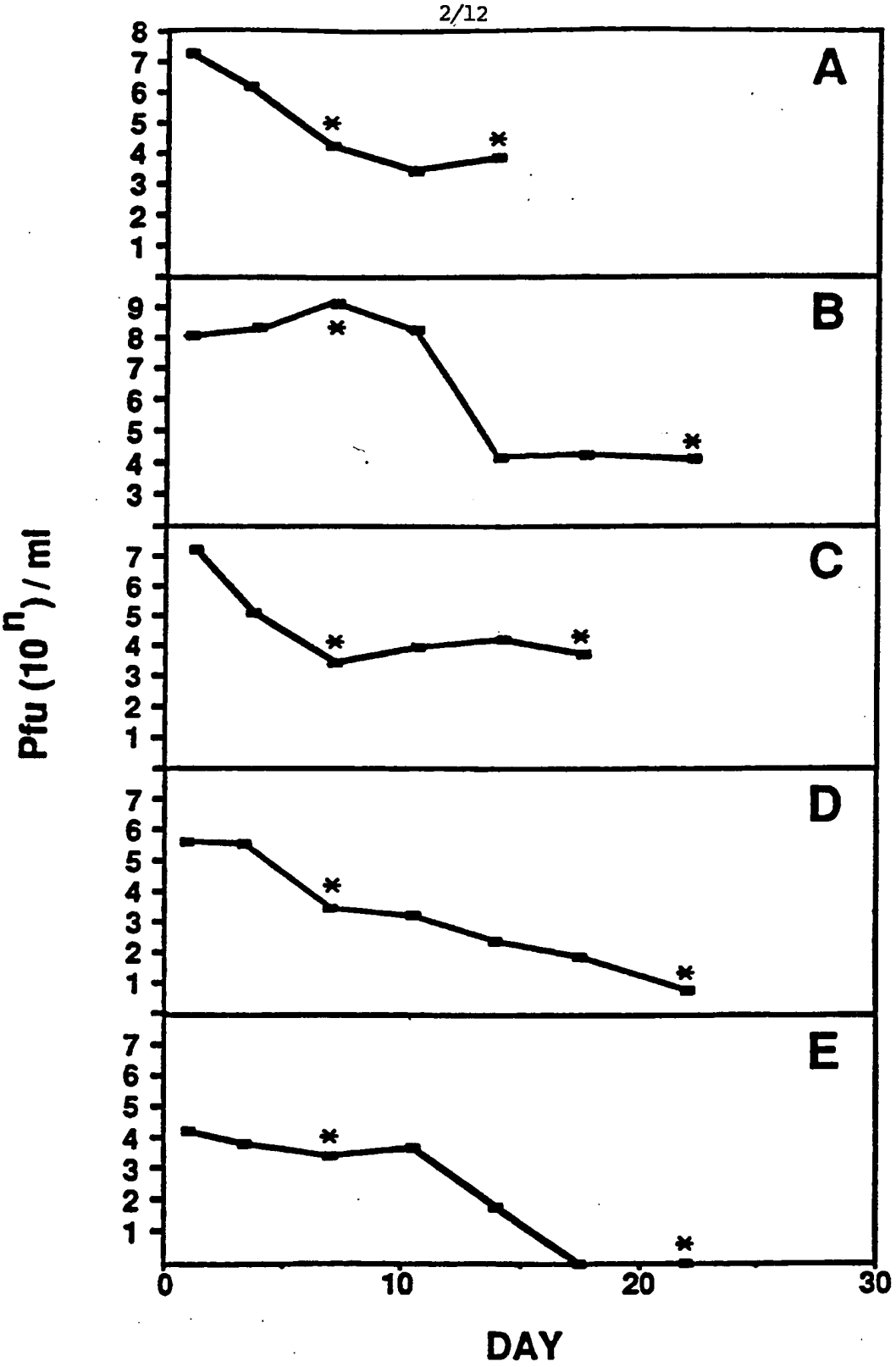
A



B



Figures 1(A-B)



Figures 2(A-E)

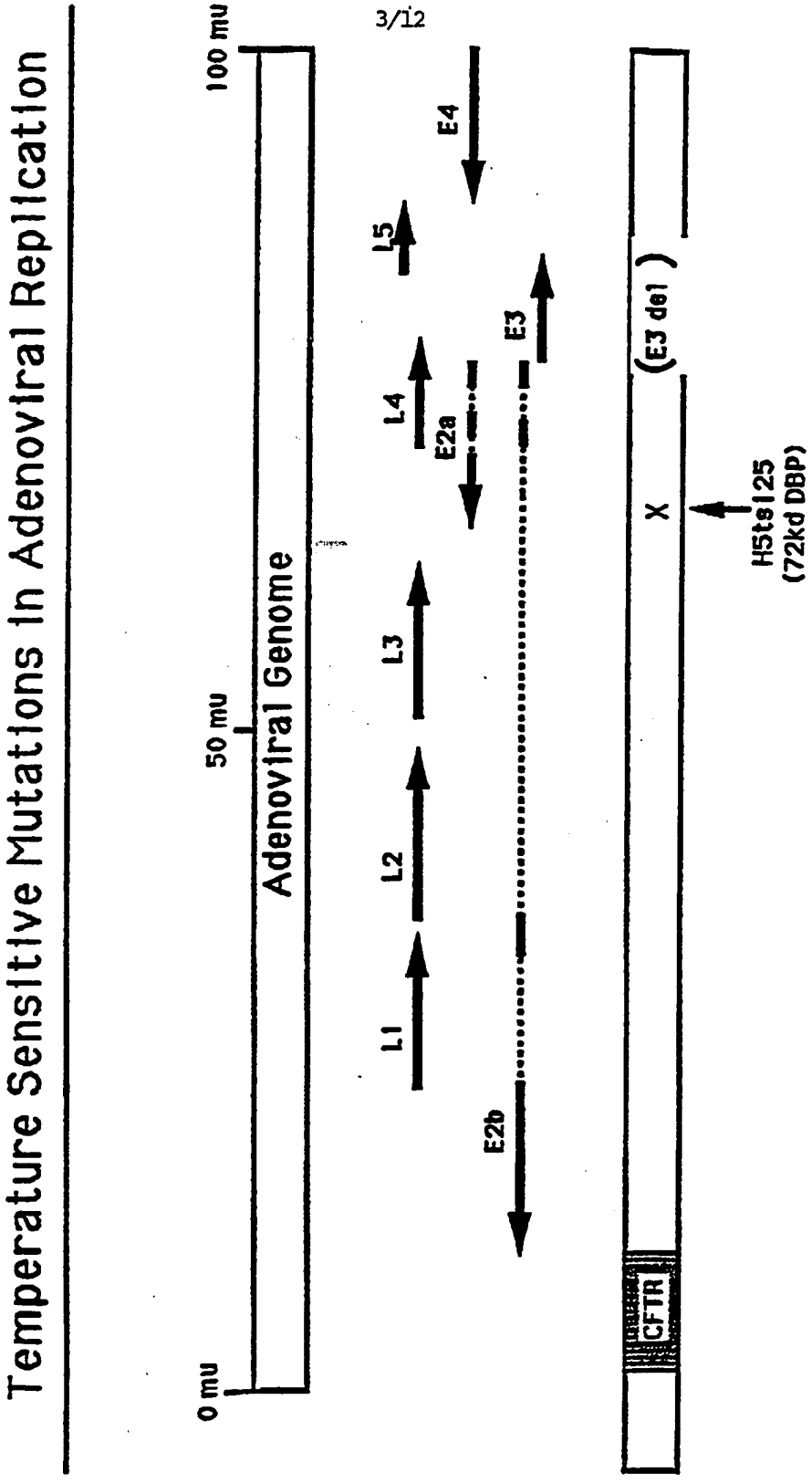

Temperature Sensitive Mutations in Adenoviral Replication
100 mu
50 mu
0 mu
Adenoviral Genome
L1
L2
L3
L4
L5
E2a
E2b
E3
E4
(E3 del)
X
H5ts125
(72kd DBP)
CFTR

Figure 3

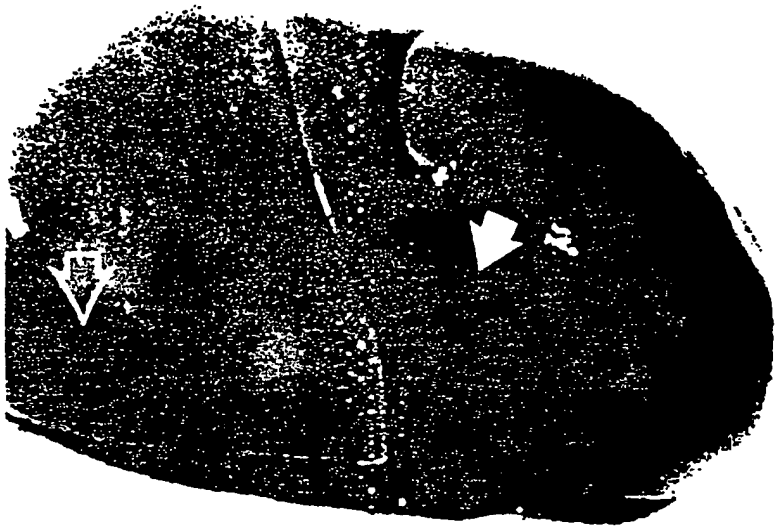
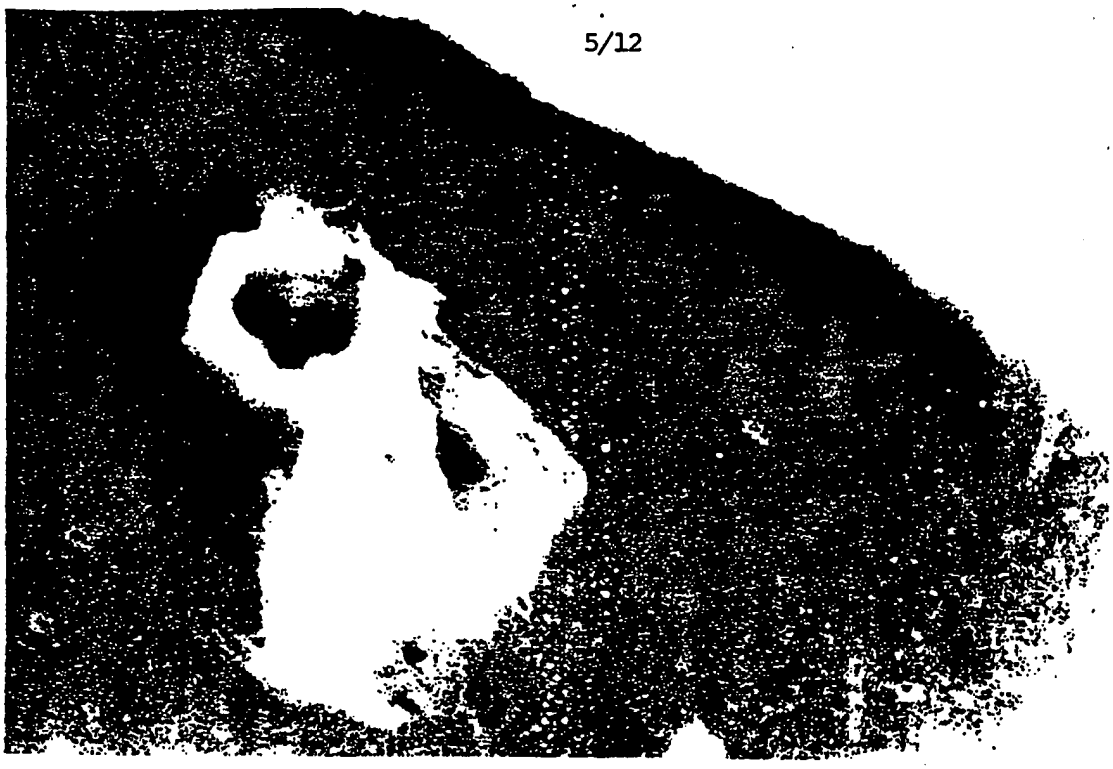
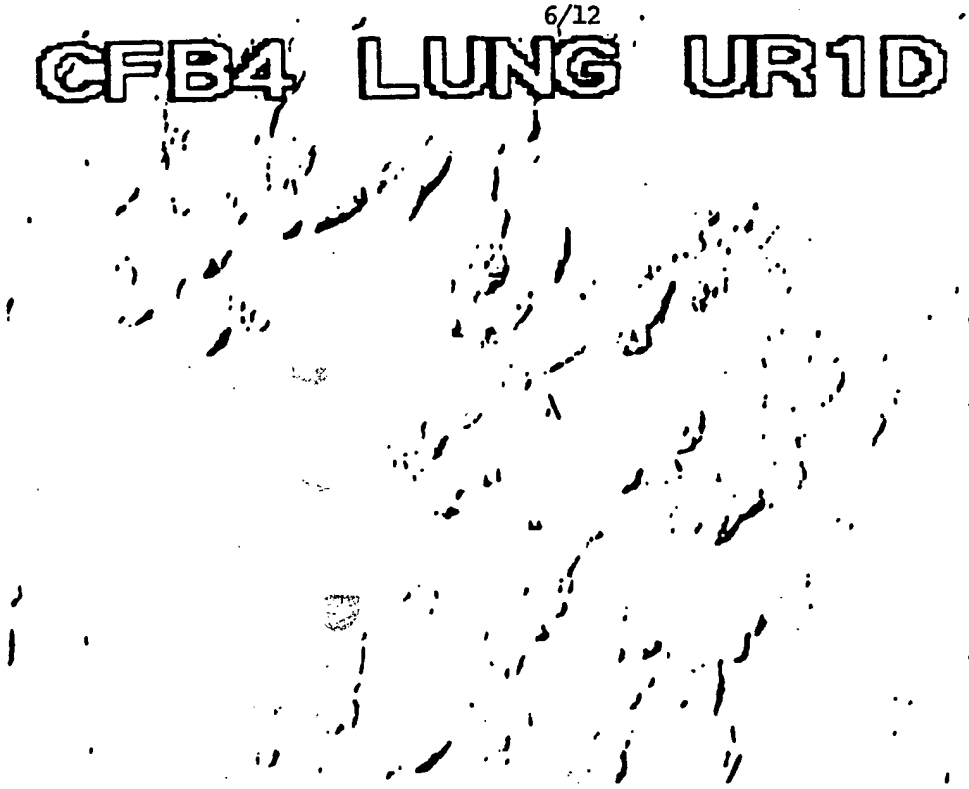


Figure 4

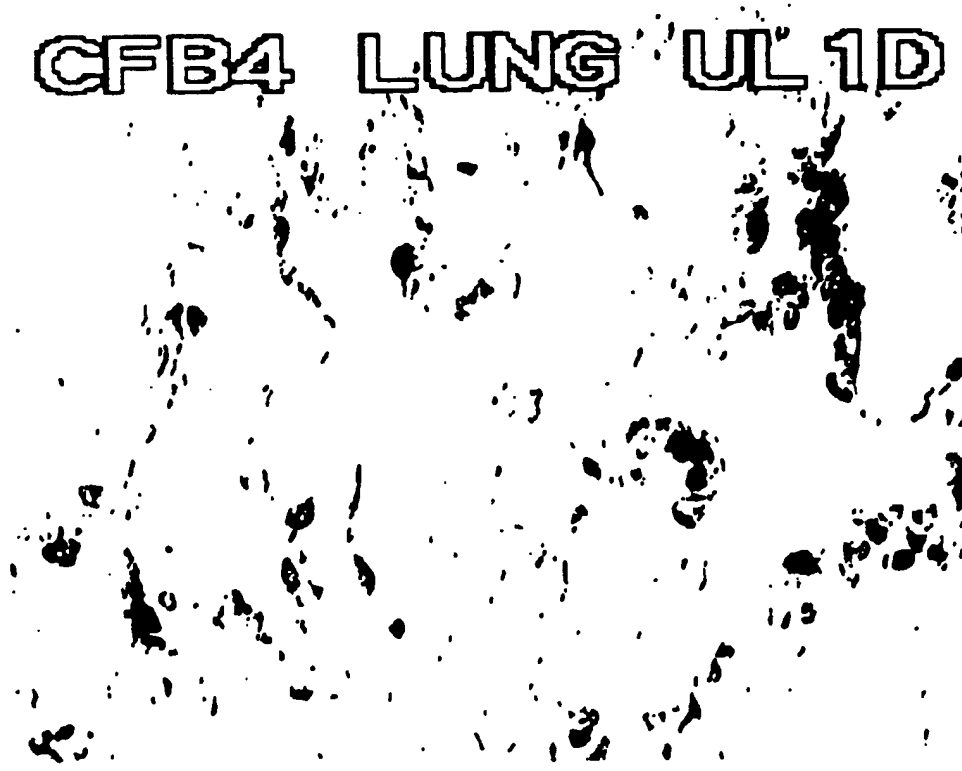


Figures 5(A-B)

A



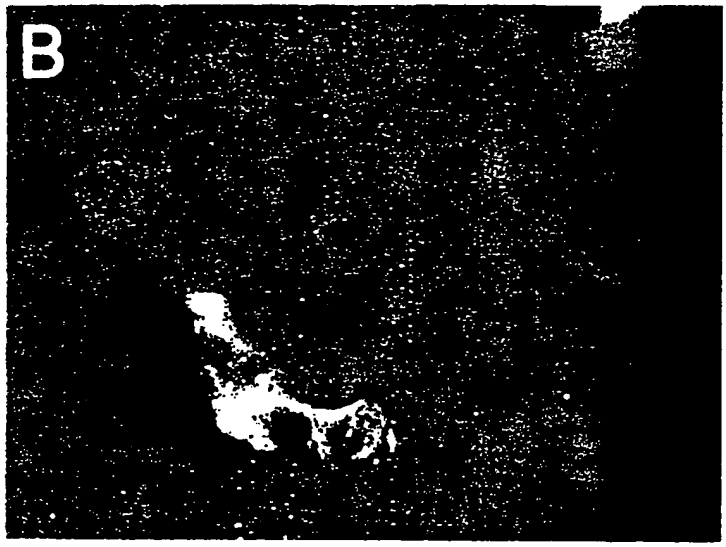
B



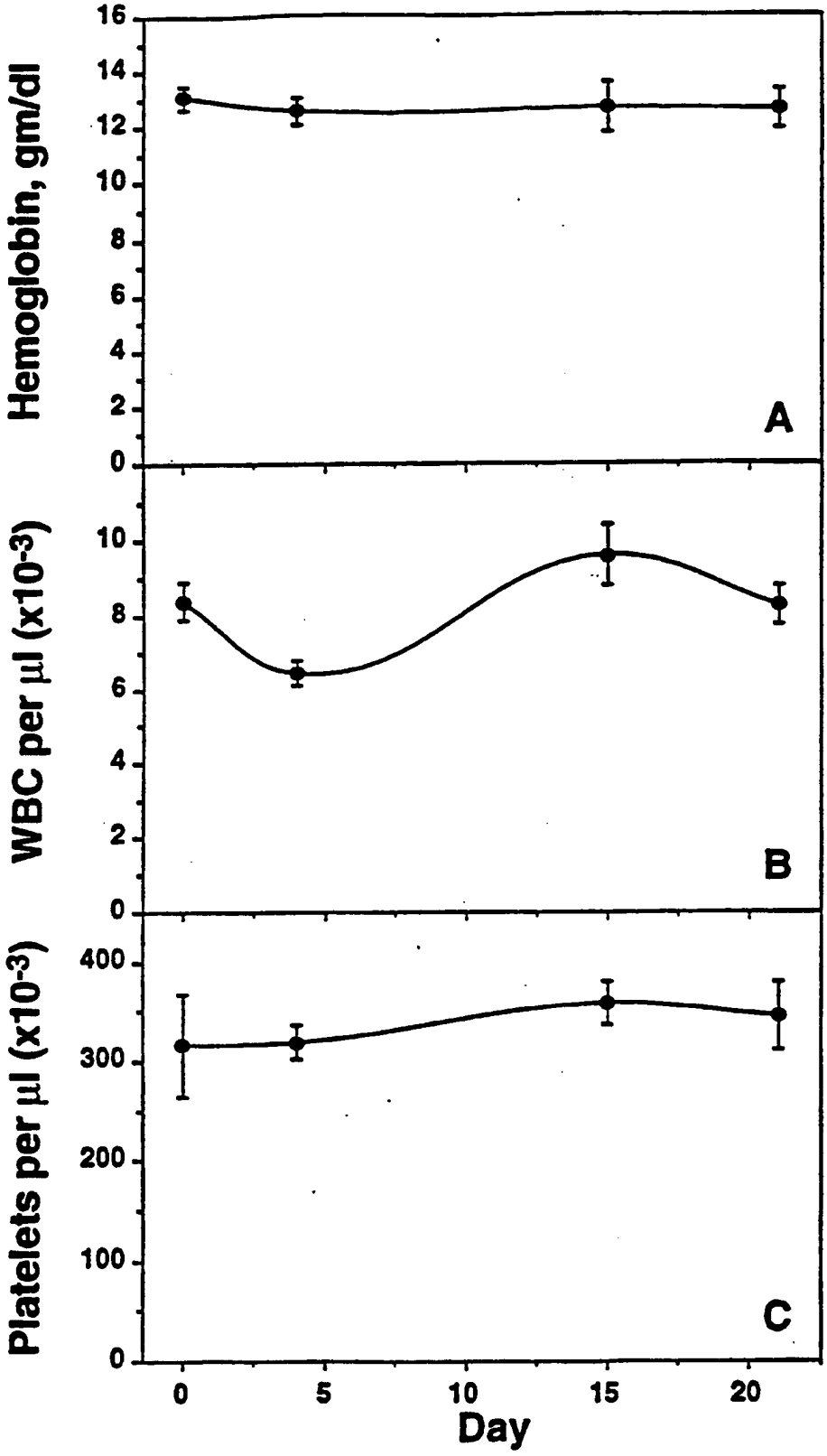
Figures 6(A-B)

A

B



Figures 7(A-B)


Hemoglobin, gm/dl
16
14
12
10
8
6
4
2
0
A
WBC per µl (x10-3)
10
8
6
4
2
0
B
Platelets per µl (x10-3)
400
300
200
100
0
C
0
5
10
15
20
Day

Figures 8(A-C)

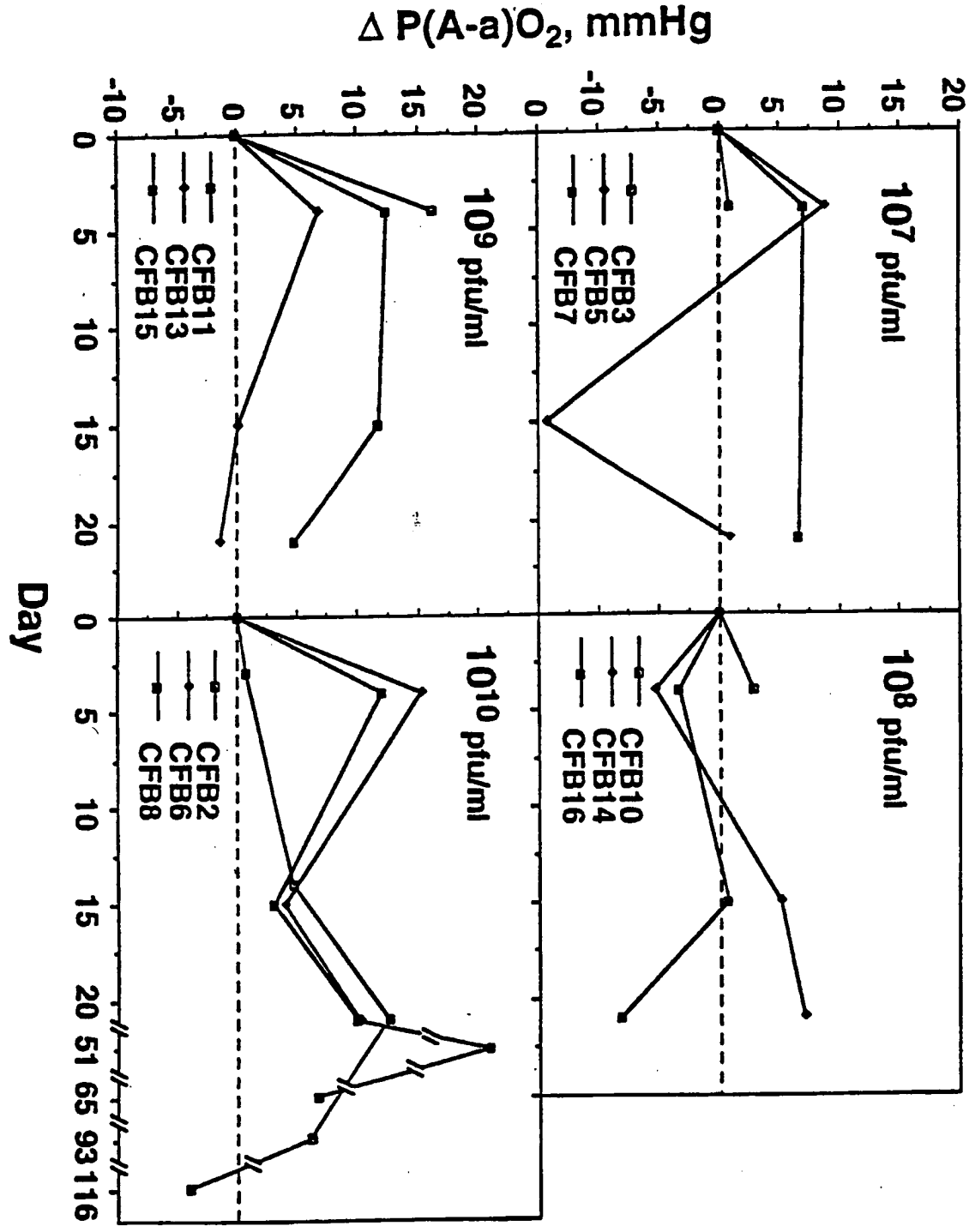

Δ P(A-a)O2, mmHg
10^7 pfu/ml
CFB3
CFB5
CFB7
10^9 pfu/ml
CFB11
CFB13
CFB15
10^8 pfu/ml
CFB10
CFB14
CFB16
10^10 pfu/ml
CFB2
CFB6
CFB8
Day

Figure 9

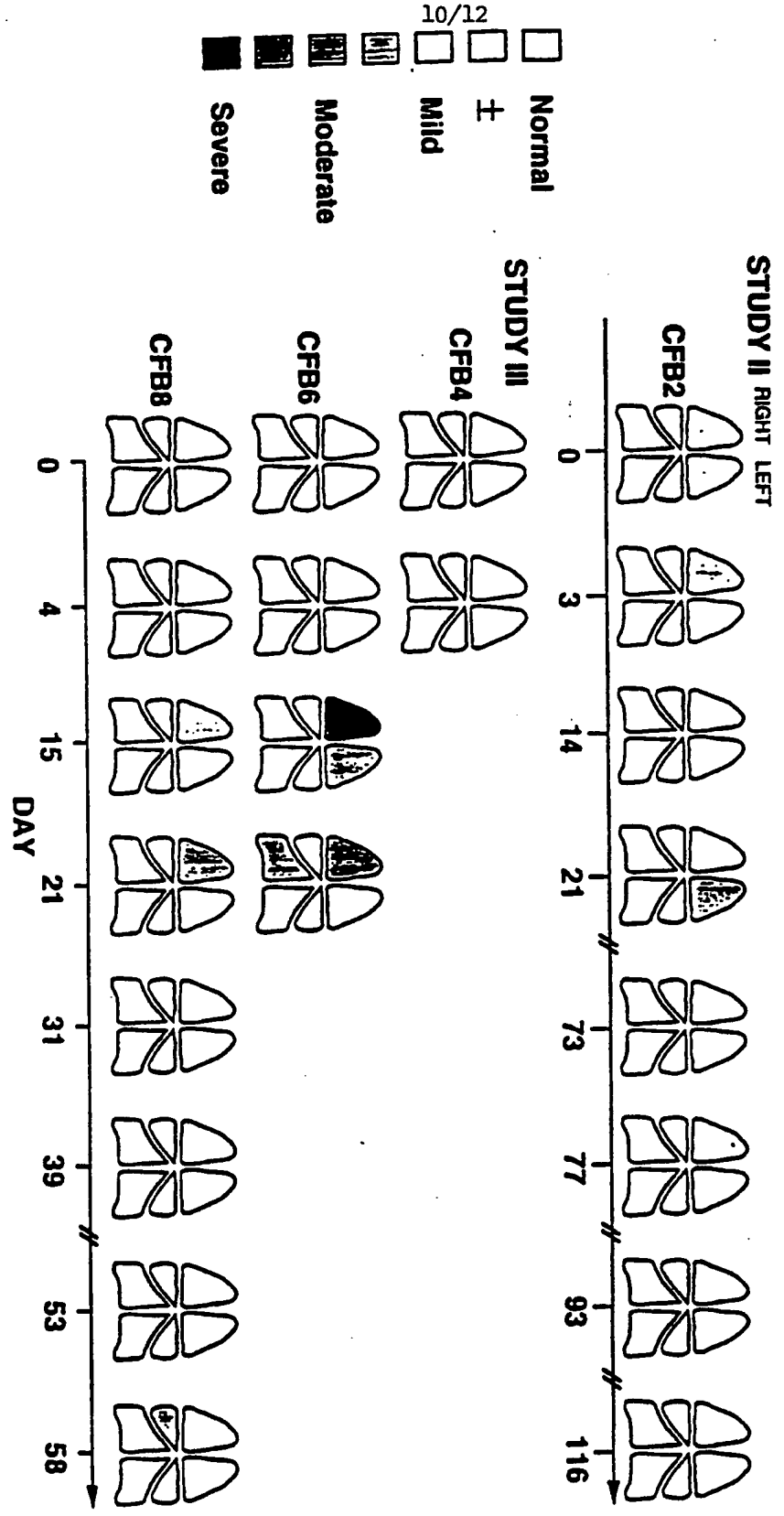

Severe
Moderate
Mild
±
Normal
STUDY II RIGHT LEFT
CFB2
STUDY III
CFB4
CFB6
CFB8
DAY
0
3
4
14
15
21
31
39
53
58
73
77
93
116

Figure 10

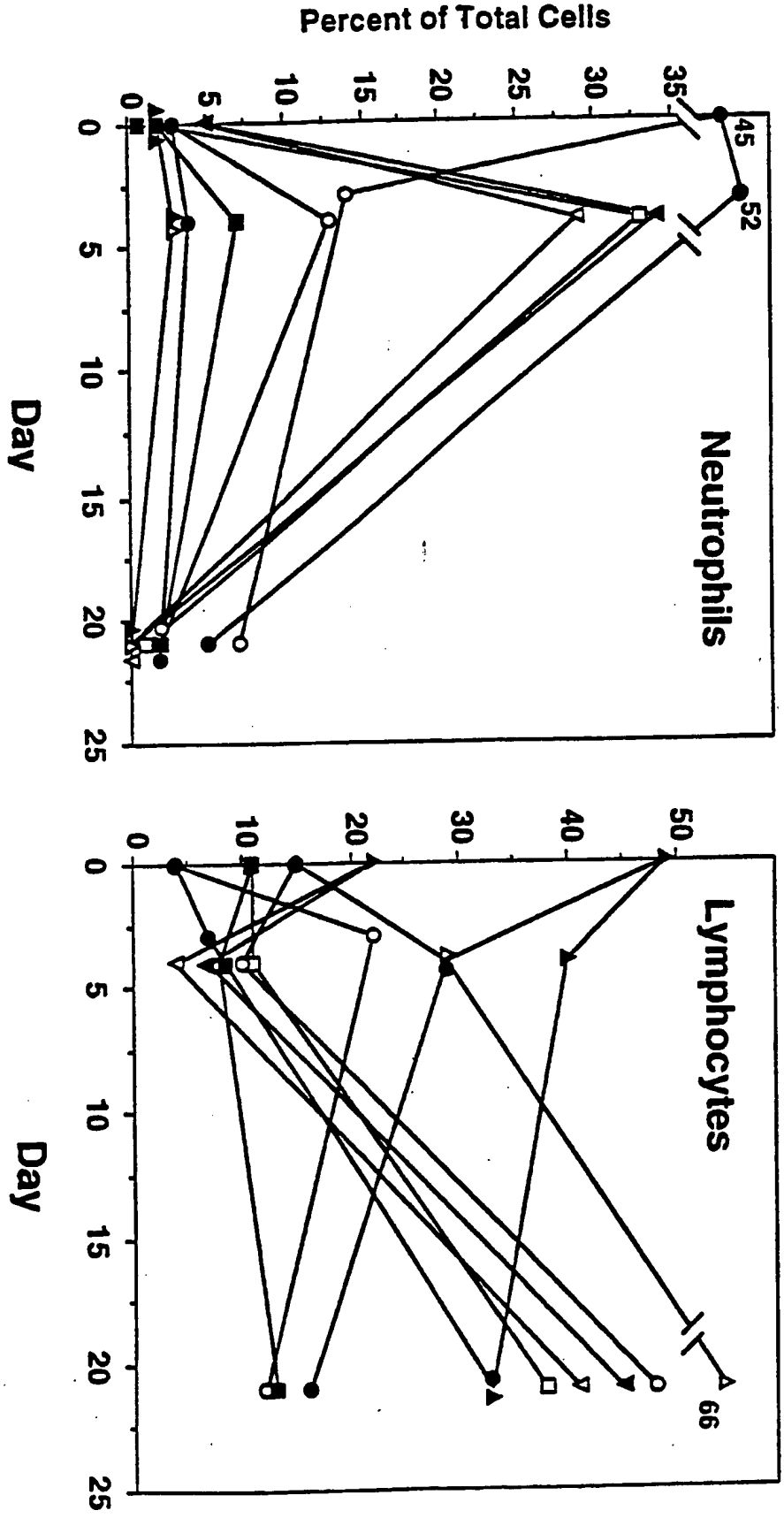

Percent of Total Cells
Neutrophils
Day
0
5
10
15
20
25
30
35
45
52
Lymphocytes
Day
0
10
20
30
40
50
66

Figure 11

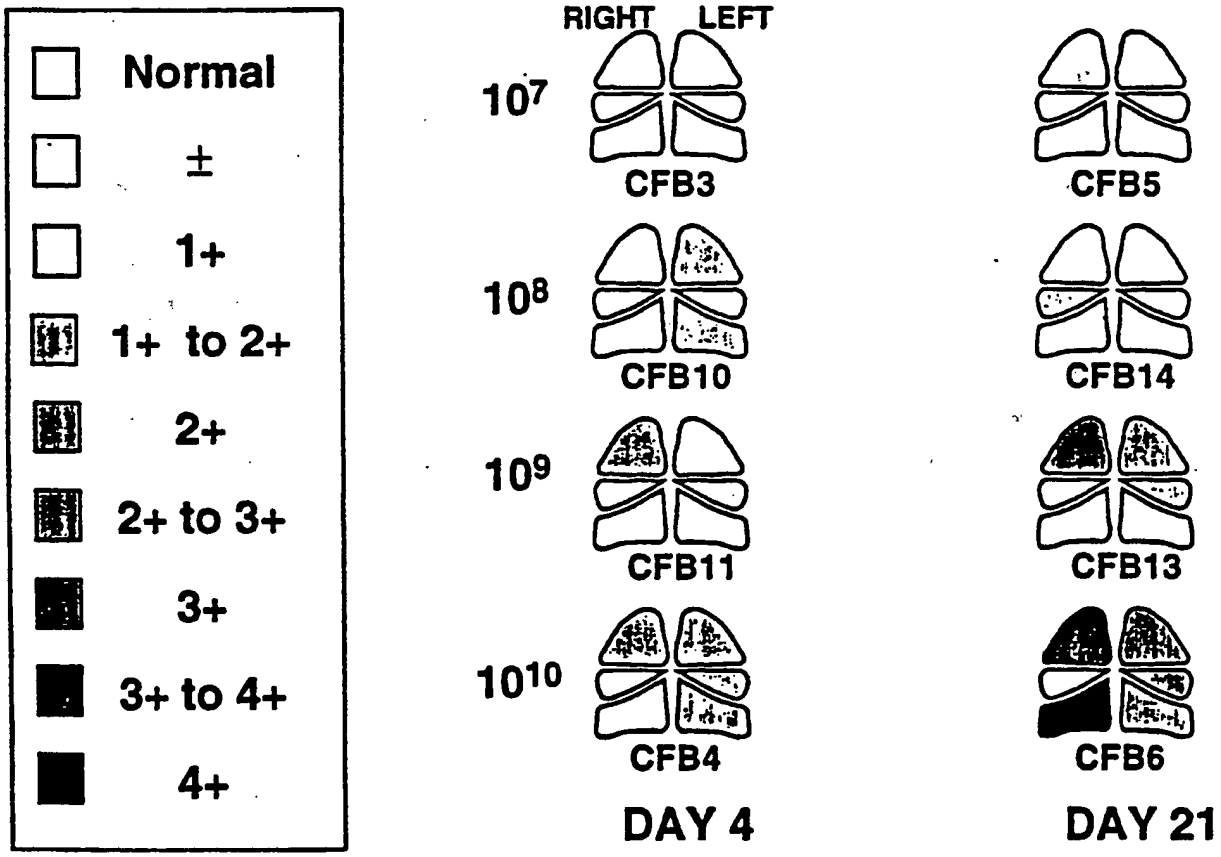

Normal
±
1+
1+ to 2+
2+
2+ to 3+
3+
3+ to 4+
4+
RIGHT
LEFT
10^7
CFB3
CFB5
10^8
CFB10
CFB14
10^9
CFB11
CFB13
10^{10}
CFB4
CFB6
DAY 4
DAY 21

Figure 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06338

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :514/44; 435/320.1, 172.1, 69.1; 935/62, 57, 32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 435/320.1, 172.1, 69.1; 935/62, 57, 32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, APS, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Virology, Volume 168, issued 1989, Spessot et al., "Cloning of the Herpes Simplex Virus ICP4 Gene in an Adenovirus Vector: Effects on Adenovirus Gene Expression and Replication", pages 378-387, see entire document.	1-5
X --- Y	Cell, Volume 68, issued 10 January 1992, Rosenfeld et al., "In Vivo Transfer of the Human Cystic Fibrosis Transmembrane Conductance Regulator Gene to the Airway Epithelium", pages 143-155, see entire document.	11-12 --- 1-10, 13-23
Y	BioTechniques, Volume 6, No. 7, issued 1988, Berkner et al., "Development of Adenovirus Vectors for the Expression of Heterologous Genes", pages 616-629, see entire document.	1-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
21 SEPTEMBER 1994

Date of mailing of the international search report
SEP 23 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
JACQUELINE STONE

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06338

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 48/00; C12N 15/63, 15/12